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I.—"SWAYBACK": A DEMYELINATING DISEASE OF LAMBS WITH AFFINITIES TO SCHILDER'S ENCEPHALITIS IN MAN

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I.—INTRODUCTION

The demyelination diseases (or myelinoclastic types of encephalitis in man) have been gradually extended in their scope. Diseases which are now embraced in this category* include acute disseminated encephalomyelitis

*See Brain and Strauss (1934); Wertham and Wertham (1934); Ferraro (1937) and Hadfield and Garrod (1938).

occurring as a primary spontaneous disease or as a post-infective complication of measles, small-pox, chicken-pox, influenza or as a post-vaccinial encephalitis; another group, more idiopathic than the above, include disseminated scelerosis, neuropticomyelitis (Devic), Schilder's disease (*encephalitis periaxialis diffusa*) and the various progressive infantile or juvenile (sometimes familial) forms of diffuse sclerosis which have been described by so many different authors under various names. To this list can be added "anti-rabic" encephalitis, sulpharsphenamine encephalitis (*e.g.*, Russell, 1937), lead encephalitis (*e.g.*, see Ferraro and Hernandez, 1932, and Blackman, 1937), a myelinopathy due to CO poisoning (Hsu and Chang, 1938), and a variety of non-systematised demyelination associated with certain nutritional disorders, *e.g.*, with pernicious anæmia and pellagra.

The essential lesion in all is demyelination, whether it occurs as scattered foci in disseminated sclerosis, as peri-vascular areas in post-vaccinial encephalitis or as massive diffuse (sometimes symmetrical) areas in Schilder's disease. This lesion is in contrast to that which occurs in any of the neurotropic virus infections (*e.g.*, in rabies, louping-ill, poliomyelitis, etc.), in which an inflammatory reaction is outstanding, in which there is a greater tendency for the process to affect the grey matter, and with which intracellular inclusion bodies are often associated—encephalitis referred to as polioclastic in type. Ferraro (1937) has attempted to unify the demyelination diseases on anatomic-pathological grounds. He classifies them into sporadic and familial forms in either of which the lesions may have a patchy or diffuse distribution, and in any of which the disease may run an acute or chronic course. He considers that in diffuse sclerosis the histo-pathological changes are fundamentally the same in all, irrespective of the name given. Any pathological or clinical differences which do occur from case to case can be reconciled with (*a*) the age of onset of the disease, (*b*) the localisation of the lesions, (*c*) the initial intensity and subsequent tempo of the process and (*d*) the constitutional make-up of the patient at the time when the causative factor began to work.

So far, all attempts to establish the ætiology of the demyelination diseases have failed; no specific organism or virus has been identified with any of them and experimental transmission has not been achieved. A neurotropic virus origin was suggested by Levaditi *et al.* (1930) but was not substantiated. No known virus acting directly on the nervous system produces demyelination (see Rivers (1932), Hurst (1936) and Ferraro (1937)).

Many theories of causation have naturally been periodically suggested without ever receiving much general support, *e.g.*, "toxins" were claimed to be a possible cause in the case of disseminated sclerosis by Dawson (1916); Glanzman (1927) and others have proposed that acute disseminated encephalomyelitis might rest on some anaphylactic basis; Putnam (1937) has suggested that the process in multiple sclerosis might rest on a vascular basis; in the case of post-infective encephalitis (after influenza, measles, etc.) and post-vaccinial encephalitis there have been contentions that the diseases were caused by the primary virus infection, or by an unknown virus empowered in some way by the exanthematous disease to attack the nervous system (see Brain and Strauss (1934)); an old idea that lead was causally connected with disseminated sclerosis was revived by Cone, Russel and Harwood (1934); Brickner (1930) and Crandall and Cherry (1931) have recently demonstrated the presence of myelin-splitting ferments in oxalated blood plasma of patients with multiple sclerosis;

Collier and Greenfield (1924) suggested that Schilder's disease might be due to a primary affection of the oligodendroglia, which cells are now regarded as influencing the nutrition of the myelin sheaths.

The ætiology of these demyelinating maladies being unknown, it must be almost impossible to assess the relationship of one to another. It is, however, highly probable that the reaction of the brain to pathogenic agents is limited, and that different causal agents can produce the same anatomical change. The demyelination problem might therefore be several, and not fundamentally one, as has been suggested by some workers.

Experimentally, tetanus toxin has been claimed to produce demyelination by Claude (1897) and Putnam, McKenna and Morrison (1931), cyanide by Ferraro (1933) and Rubino (1935), CO poisoning by Meyer (1928), saponin by Cornil, Poursines, Giraud-Costa (1938); Rivers, Sprunt and Berry (1933), and Rivers and Schwenkter (1935) have claimed to produce demyelination in monkeys by injections, over a prolonged period, of emulsions of heterologous brain substance.* Little or no attempt has been made to follow up any of these experimental methods, and the mechanism involved in demyelination is still not understood. Any approach to the problem, other than by a study of the end stages of the human disease, has therefore not been highly productive of results, and the present position might almost be regarded as one of stalemate.

The above is a brief summary of the problem as it affects the human being, and we may now turn to a consideration of analogous spontaneous conditions in animals.

A disease resembling Schilder's encephalitis has been described in monkeys by Perdrau (1930), Levaditi *et al.* (1930 and 1933), Schob (1931), Scherer (1932), Davison (1933-34), and Hurst (1937); apart from the isolated claim of Levaditi, in none of these cases were transmission experiments successful. The ætiology thus rests on the same obscure basis as that of the human diseases.

A type of encephalo-myelitis in dogs (usually referred to as " post-distemper " encephalitis) was described by Perdrau and Pugh (1930) in which demyelination, of a type commonly found in subacute disseminated sclerosis of man, was seen in four out of fourteen cases. Many veterinary authorities still believe that this encephalitis is a true post-distemper complication, although no connection was established by Perdrau between the encephalitis and the distemper virus. From an examination of nearly 50 cases and from some preliminary transmission experiments, our own work (Innes and Dalling), on the same subject, confirms much of Perdrau and Pugh's findings. Distemper virus cannot be identified from the brain or spleen of such cases, nor can a true encephalomyelitis be set up by intra-cerebral inoculations of the virus. The ætiology, therefore, rests on the same uncertain basis as the post-infective or post-exanthematous demyelinating diseases of man.

An " anti-rabic encephalitis " also occurs in dogs, Mócsy (1937) which, as in man, demands an explanation of its relationship to the rabies virus.

Finally, there is this disease in lambs referred to as " Swayback," which has attracted attention only recently. We regard " Swayback " as being

*A similar series of experiments was carried out by one of us (J.R.M.I.) using ewes and lambs instead of monkeys. Both ewes and young lambs were injected thrice weekly with heterologous (rabbit) brain emulsion for a period of one year with completely negative results.

the nearest analogy in domestic animal pathology to the more idiopathic demyelination diseases of man, and in particular to Schilder's encephalitis. The problems of its ætiology must be regarded therefore as having a bearing on those of the human maladies. A preliminary description regarding the pathology was made by Innes (1936). Much work has been done since then, but as a later paper was published only in an Institute Report (1936) it is appropriate now to record the sum total of our present knowledge concerning this disease.

II.—History and Geographical Distribution of "Swayback."

"Swayback," Swingleback," "Swingback," "Jinkback," "Jingleback," "Bentback," "Evil," "Warfa" (Derbyshire), "Belland" (Derbyshire), and "Bride" or "Cefn-Gwan" (Wales) are the names which have been given, probably by shepherds in different localities, to this disease of young lambs; some of them presumably on account of the outstanding symptom, namely, the inco-ordination. While records of this disease have only appeared in the literature recently, there is plausible evidence that it has been known in England for generations.

It may appear peculiar that the disease should have evaded description by early veterinary writers. Some of the clinical descriptions of lamb paralysis, however, may well apply to those of "Swayback," *e.g.*, see Steel (1890). The classical writings of the Ettrick shepherd (Hogg, 1807) contain no description of any condition which might be comparable.

It appears similar to a disease of lambs in Peru, investigated by Gaiger (1917), called "renguerra"—a word of Spanish origin referring to an "injured back" (or with a Creole origin meaning to wobble or sway). This disease was (and still is) well-known to the sheep farmers there and was considered responsible for annual losses of several thousands of lambs. Symptoms of paralysis were observed in lambs between two and six weeks old. Most of the lambs died but some of the more slightly affected cases survived. Gaiger was apparently then aware of a similar disease in Britain as he stated that "Renguerra" bore some resemblance to "Swingback," but because little was known at that time about nervous disorders in sheep (*viz.*, louping-ill, scrapie and "Swayback"), it was uncertain how far this similarity existed. He also mentioned that "renguerra" compared closely with descriptions of a nervous disorder in lambs called "pataleta," which occurred in Argentine and Patagonia. The pathology of "renguerra" was not investigated and the ætiology not determined. There have been apparently severe periodic outbreaks of "renguerra" from 1911 onwards extending over two or three lambing seasons, followed by a period of relative freedom—a feature not unlike that met with in England.

Magnusson (1920) recorded a disease in Sweden which appears similar to "Swayback." Young lambs between a week and a month

old were affected with a limb paralysis; although macroscopic lesions were said to be absent from the nervous system, the pathology was not determined. Attempts to identify either a causal organism, virus, or any other ætiological factor, failed. He also referred to the clinical resemblance between the Swedish disease, louping-ill, " Swingback " and scrapie in Britain.

The first record of the disease in Britain was by Lyle Stewart (1932). Bacteriological examinations of two cases were made with negative results. A pathological examination of the brain was not made, but Lyle Stewart demonstrated degeneration in the ventro-lateral columns of the spinal cord. It was concluded, however, that this represented a degeneration of the ascending tracts. The ætiology was not determined.

A disease of young lambs was described by Bennetts (1932) in Australia and called " enzootic ataxia." He also observed degeneration in the cord and came to the conclusion that it was of an ascending type. No mention was made regarding any examination of the brain, and the pathology was left as obscure. Brief communications were subsequently made by Dunning (1933) from South Africa*, and Krishnappa (1936) from India, suggesting that a " Swayback-like " condition in lambs existed in these countries.

In spite of the absence of accurate pathological data the descriptive similarity of the history, symptoms and age incidence of affected lambs, might indicate that a disease comparable to " Swayback " has been known to occur in South America (Peru, Patagonia and Argentine), Sweden, India, South Africa*, Australia and New Zealand.† As a result of personal enquiry, it appears that no comparable nervous disorder of lambs has been observed in Germany.

III.—Occurrence, Incidence and Field Aspects of the Disease in Britain.

The attention of workers in Cambridge was first attracted to the subject in 1926 by reports of sporadic outbreaks of paralysis in new-born or young lambs, from apparently healthy ewes, in various parts of the country. Attempts to isolate a causal organism or to transmit the disease to healthy animals failed, and no further serious work was done until 1935. Since then, investigations have been made both in the field and laboratory, and much new information obtained which has caused the problem to be viewed in an entirely new light.

The disease appears to have a wide geographical distribution throughout England, Wales and Scotland. Outbreaks have either been personally investigated (or cases received from outbreaks) in Derbyshire, Buckinghamshire, Northamptonshire, Worcestershire,

*Dr. Du Toit, Onderstepoort, has informed us that the disease, if it occurs in South Africa at all, is certainly not widely known.

†Personal communication by Mr. C. S. M. Hopkirk, New Zealand.

Leicestershire, Bedfordshire, Gloucestershire, Wiltshire, Yorkshire, Herefordshire, Suffolk, Lincolnshire, Rutlandshire, Warwickshire, Somerset, and in several counties in Wales—Glamorgan, Breconshire, Denbighshire, Merioneth, Montgomery, North Cardigan. These data have been substantiated by Roberts (1938), who also recorded the occurrence of " Swayback " in Scotland in the Island of Cathay, Caithness and the South-West and Border counties. The disease has been reported in other counties, *e.g.*, Kent and Cumberland.

The incidence varies from year to year on the same farms and in the same districts; it seldom, if ever, occurs only on any one farm in a district, *i.e.*, there is always an area involved. While the disease has a wide distribution throughout the country, with an irregular annual incidence, there are districts in which it occurs with disastrous periodicity, *i.e.*, the disease might be regarded as an enzootic in these areas. The incidence in other parts of the country may be very low, the farmer losing only one or two lambs annually. According to Roberts (1938) this is a common experience in Scotland. In the more severely affected areas the mortality may vary annually from 1 to 50 per cent. of the lambs born, while in exceptionally bad years the losses may be even 90 per cent.

On some farms outbreaks have occurred each lambing season for many years, while on others it was observed for the first time in 1935, *i.e.*, when these investigations were begun. Further enquiries have revealed comparable instances of the disease occurring on farms after many years freedom. On the other hand, the disease has apparently disappeared from certain areas in which it was prevalent in 1935, *e.g.*, on certain farms in Leicestershire. Many farmers believe that the prevalence has increased recently; it is impossible to confirm or refute this belief, as until 1935 almost no attention was given to the problem.

Another belief that the progeny of Border-Leicester ewes were more susceptible than other breeds was disproved by our own observations. " Swayback " has been seen in lambs of all the well-known breeds, *e.g.*, Suffolk, Masham, Wensleydale Cross, Southdown, Welsh Mountain, Blackface, Kerry, Exmoor, Swaledale, Gritstone, Woodland, Baumshire, etc., and in many of these breeds crossed with Oxford, Suffolk, Hampshire and Border-Leicester rams.

Ewes of any age may give birth to affected lambs, but there was a suggestion that the older ewes were more prone to do so. For example, the records of the disease on one farm in 1934 indicated that the majority of cases were lambs from ewes which had had three to four previous gestations in that area. In the 52 recorded cases that year only two were from first-lamb ewes. This suggestion is confirmed by some farmers and mooted by others in different parts of the country. Further personal experience has indicated that in a large number of cases, old ewes, or ewes which have been in an " affected " area more than one year are more prone to give birth to

" Swayback " lambs. We have, however, seen cases from ewes which have been kept on an " affected " farm for a period of only six months.

Ewes which have given birth to " Swayback " lambs one year may produce either affected or healthy lambs the following season. Either sex, single lambs, both of twins and all three of triplets may be diseased with no proclivity for any of these. Rarely only one of twin lambs was observed to be affected at birth, but in such cases the other often showed symptoms later in life. When only one of twin lambs is visibly affected at birth we do not know whether the other lamb remains permanently free from symptoms.

In our own experience most of the cases have been affected at birth but some do not show symptoms (or are not observed) until they are a few weeks old, at the latest about six weeks. (In Derbyshire it is said that some cases are not affected until they are two months old, and then get gradually worse. We were unable to examine any of these lambs and are unsatisfied that they are true cases of " Swayback "). Earlier unrecorded experiences suggested that the disease was more often of this latter type and that in recent years there had been a greater incidence of newly-born lambs affected. In any one outbreak both forms are usually encountered.

Observations in an Enzootic Area in Derbyshire.

A field investigation was begun in 1938. All the information given in the previous section regarding the disease elsewhere in England is applicable to the condition in this area.

" Swayback " has been known to the farmers for generations, almost certainly over a hundred years. A total of 79 farms were included in this survey which covered an area of 38 square miles with the village of Bradwell in the centre.

The losses from " Swayback " have been so severe in the past that many farmers have been compelled to give up sheep breeding or the farms have been actually vacated. Out of 3,030 lambs born in 1938 there were 315 cases of " Swayback," *i.e.,* 10 per cent., compared with an estimated 15 per cent. mortality in the same area in 1937; the incidence on the different farms varied from 1 to 72 per cent. This variation in different farms in the same district in any one year is a common finding in other parts of England. These figures are unassailable evidence that " Swayback " is the most serious disease affecting sheep in this area.

The disease occurs on farms adjacent to others on which the disease is not known, but some of the owners follow a practice of wintering the ewes away from the farm and this is suggested as a reason for the absence of " Swayback."

The incidence in this Derbyshire area is also said to vary from year to year, depending on the amount of winter and spring herbage consumed by the ewes. If large amounts of hay and concentrates have to be fed to the pregnant ewes on account of a severe winter the incidence is said to be reduced. The disease is also said to be prevented by transferring the ewes to " unaffected " areas (mainly to Cheshire and Yorkshire) during pregnancy, from about November until lambing time, and many of the farmers

in this area practise this method of "prophylaxis." This experience is also quoted by farmers in other areas and by Bennetts (1932) in connection with the Australian disease.

IV.—Signs and Symptoms.

Whether the animal is affected when born or not until later, the symptoms are essentially the same, varying only in degree of severity (see Figs. 1 to 6). All cases show inco-ordination of movement; some are totally unable to stand or walk and lie helpless (Fig. 2); others may rise with difficulty to collapse almost immediately (Fig. 5); others may walk with a very straddled, staggering gait (Fig. 3), while mild cases show only a slight weakness of the hind quarters, particularly when hustled or made to turn quickly. Although this ataxia is always definite, and often severe, there is no flaccidity; on the contrary, spasticity may be marked. Fits are not observed. The flexor reflexes and knee jerks are brisk, but not exaggerated; tremors are inconstant and the head-and-neck righting reflexes appear to be unaffected. While the corneal and pupillary reflexes are normal, many of the more severely affected cases are blind. The disease is non-febrile and remissions do not occur.

The most severe cases are those which show flagrant symptoms at birth, the longer the period which elapses from birth until the onset of symptoms resulting in a milder type of disease. The mildest cases, usually first observed some days or even weeks after birth, may show only slight unsteadiness when walking, and this inco-ordination may never progress. Such animals may survive, and when bred from later may give birth to healthy lambs.

Some of the worst cases were artificially fed and kept under observation, but the symptoms progressed and death occurred in a few days to a few weeks from broncho-pneumonia, some other infection, or simply from marasmus. Under natural conditions the survival period is much shorter owing to neglect, exposure, and/or malnutrition and the mortality of these severe cases must be about 100 per cent. As a result, these acute cases are usually killed by the farmer as soon as they are observed.

The essential manifestations of the disease are thus a spastic paralysis, particularly of the hind limbs, sometimes blindness, and a progressive (non-febrile) course with a generally fatal termination, except for the mild cases mentioned.

V.—Pathology.

A total of 167 cases have been autopsied during the last five years. These included cases sent to the Institute alive or dead and others examined during field investigations. In addition, the head or brain alone from many other lambs has also been examined. The cases were obtained from many different outbreaks in various parts of the country, and included some from the same flock or ewe in successive years. Some of the cases

were killed for immediate autopsy while others were kept under observation until death. It was thus possible to examine affected lambs of ages varying from birth to many weeks. All cases were autopsied in the usual routine manner. The viscera from a small number and the nervous system of nearly 50 cases were subsequently examined histologically. After appropriate fixation in the case of the nervous system, routine neuro-histological technique was utilised on frozen, paraffin or celloidin sections for the demonstration of myelin sheaths, axis cylinders, nerve cells and neuroglia. Other examinations which were carried out are mentioned later in appropriate parts of the paper.

(1) Macroscopic.

No significant lesions were found in the visceral organs of any case—the changes were confined to the nervous system. Muscular atrophy was never observed.

In those cases which died from some secondary complication, broncopneumonia, pleurisy, and occasionally a purulent meningitis, appeared to be the commonest causes of death.

Nervous System.—Gross changes were present in the brain in 101 out of 167 cases examined. The different macroscopic appearances indicated that any large series such as this gave a good representation of the gross genesis of the lesions.

The most severe or acute type of change seen may be described as follows: The bones of the cranium were thin and easily cut, but with the sutures united. On opening the calvarium a large quantity of clear cerebro-spinal fluid flowed out from dilated subarachnoid spaces. There was thus an obvious "retraction" or atrophy" of the brain from the skull, a fact substantiated by X-ray examination of a detached head. The brain as a whole looked like a water-logged organ. The convolutions of the cerebrum appeared poorly developed or were ballooned out by fluid within, so that the sulci were shallow and poorly defined. Fluctuation could be felt in the brain, and it was clear that the cerebral cortex consisted only of a thin shell of substance. The latter was sodden, felt flabby, and without care laceration caused collapse of the cerebral hemispheres, and gave the latter the appearance of deflated balloons (Fig. 8). No lesions were observed in the meninges (except in two cases which showed a secondary purulent basal meningitis) and there were no marked vascular changes in any part of the brain. Lesions were not found in the cerebellum, hypophysis, mid-brain, brain-stem, chorioid plexuses of fourth ventricle, spinal cord throughout its length, posterior root ganglia or peripheral nerves—the latter in many cases being dissected to their termination. After fixation, the brains were cut either into horizontal or coronal sections to disclose a diffuse symmetrical degeneration of the cerebral white matter. The latter was replaced by a loosely woven, greyish, gelatinous substance or had been completely liquefied, as was evident by the presence of gross cavitation (Figs. 5, 7, 9, 10, 18). The transition between the gelatinous degeneration and liquefaction was often apparent at different levels of the hemispheres. In the most severe cases this porencephalic-like cavitation extended from the occipital to the frontal poles (Figs. 5, 18). The central white matter was thus restricted in most cases of this series to a grossly degenerated *centrum* ovale, to a wasted *corpus callosum* and *septum pellucidum* and to the internal capsules. A *cavum septi pellucidi* was frequently noticed. The cerebral grey matter

was relatively well-preserved but formed only a thin shell around the degenerated white matter or the cavities (Figs. 5, 7). There was a slight to moderate internal hydrocephalus of the lateral ventricles in many of these severe cases (Fig. 5); the ventricular walls were smooth but communications with the cavities had been often effected by small perforations. The aqueduct was rarely dilated and the fourth ventricle never so. The basal ganglia, the thalamus, mid-brain, pons, cerebellum, medulla and spinal cord (at all levels) were normal. Gross cerebral changes, as described above, were observed in stillborn lambs (five cases), in lambs killed a few minutes after birth and in animals four to five weeks old.

In 29 out of these 101 lambs showing gross changes, cerebral lesions were obviously in an earlier stage of development. All gradations were observed in the different cases, ranging from small foci of gelatinous softening of the white matter, to more diffuse areas involving the entire occipital lobes, and to symmetrical gelatinous degeneration of the whole hemispheres (Fig. 15). The brain always felt soft and pulpy; demarcation between the grey and white matter was extremely blurred, and cavity formation was of a minimal degree or completely absent (Figs. 15, 16). Where only the occipital poles appeared to be affected, on coronal sectioning the changes diminished in intensity as the frontal region was approached until a level might be reached which appeared normal. Ventricular dilation was rarely observed. In only two cases were the lesions unilateral (Fig. 9).

In 66 of the 167 cases no abnormalities were observed by the naked eye in any part of the nervous system. The grey and white matter throughout was clearly demarcated, well preserved and apparently normal in amount and distribution (Fig. 14).

(2) Microscopic.

No significant lesions were observed in the abdominal and thoracic visceral organs of the animals examined, apart from a few cases in which the liver and kidney showed a marked lipoid degeneration. The meaning of this lesion was obscure, but owing to its inconstancy it could not have had any direct connection with the primary nervous disorder.

Nervous System.—Diffuse, focal, extra-adventitial, or perivascular infiltrations by inflammatory cells were never observed in any part. Similar negative findings were obtained in regard to hæmorrhages, vascular thrombi, pia-arachnoid changes, pigment deposition and the structure of the chorioid plexuses. Occasionally, the perivascular spaces of cerebral vessels in the severe types of disease were dilated, no doubt due to distension by the increased fluid present in cavities, ventricles and subarachnoid spaces.

All the preparations stained by Weigert-Pal and/or Loyez's method, etc., for myelin were compared with corresponding sections from control lambs' brains (foetal, a few days and a few weeks old) (Figs. 18 to 25).

Demyelination appeared to be the constant, and in all probability the primary lesion encountered. The most severe type of change was represented by an almost total loss of myelin in the cerebral hemispheres (Fig. 21). The lesion was symmetrical, not sharply defined, and affected all zones of the white matter diffusely, to the summits of the convolutions. All gradations from this complete destruction of cerebral myelin to small foci were observed in the series of cases examined (Fig. 27). Where macroscopic

cavitation had been prominent, the white matter was grossly wasted or had completely vanished, and the persisting remnants formed at the most a thin subcortical zone or an inner wall of the cavities. Under high magnification, beading and fragmentation of the sheaths was quite evident where demyelination was in an early stage. In some of the cases in which no naked eye changes had been observed, small foci of demyelination were visible in Weigert-Pal preparations, often confined to the *centrum ovale* of the occipital lobes. These were not sharply defined and were not restricted to perivascular areas. In other cases of the same type it was often difficult to determine demyelination by the Weigert-Pal or Loyez method; this was perhaps due to the extreme "scattering" of degenerated fibres, as Marchi-Busch preparations showed that degenerated fibres were present.

In the Marchi-Busch preparations of areas adjacent to those which showed unmistakable demyelination by the Weigert-Pal method, accumulations of stainable lipoid were present (Fig. 28). Frozen sections of the same areas stained by Scharlach R also gave a positive reaction for lipoid. It was, however, surprising to note that these lipoid by-products of myelin destruction (stainable by Herxheimer and Marchi) were never abundant—borne out by the paucity of the amoeboid phagocytic glia.

In preparations stained for axis cylinders it was obvious that these structures had been destroyed as quickly as the myelin sheaths since no stainable structures were seen at all in some areas. The white matter appeared to be broken up with such rapidity that it was impossible to determine whether or not we were dealing with a primary neurolytic process with secondary affection of the axis cylinders.

Nissl and hæmatoxylin-eosin preparations of different cases showed all gradations of loss of tissue coherence of the white matter, from an apparently normal compact appearance to an extremely spongy state with a few scattered nuclei and finally to complete disappearance with porous areas and cavitation. There were no collections of cells present which are characteristic for known infections (Figs. 29 and 30).

No gross patches of demyelination were observed in the capsules, mid-brain, cerebellum and mid-brain stem by Weigert-Pal methods (Figs. 22 to 25).

In Marchi-Busch preparations degenerated fibres could occasionally be traced through the capsules, mid-brain to medulla and cord. Scattering, however, made it extremely difficult to follow these with ease, and it was not until the lower level of the medulla (above the decussation) was reached that grouping of degenerated fibres into definable tract systems was unmistakable. In the upper part of the cord (transverse sections) the degeneration was symmetrical and restricted mainly to (*a*) the ventral part of the ventro-lateral column of white matter applied close to the median fissure and reaching to the surface and (*b*) the dorsal part of the same column extending from the tip of the dorsal horn of grey matter to the surface below the dorsal root (Figs. 33, 34). Scattering of degenerated fibres was, however, a constant feature throughout the ventral white columns. Prominent degeneration was never constantly observed in any part of the ascending columns. The degeneration in the ventro-lateral columns was constant and obvious in all Marchi preparations, but by Weigert-Pal it was only visible as a paling of the areas concerned (Fig. 35). It was never seen as solid blocks such as might be seen in certain secondary degenerations of the human cord. This feature may have been due to the fact that the

degeneration was being observed at a relatively early stage with no sclerosis, and also because the fibre tracts of the sheep normally do not form such group systems as in man. This degeneration was traced to lower levels of the spinal cord without any alteration in anatomical location (Fig. 34).

The relative resistance of the nerve cells in all parts of the system was an outstanding feature, and it was clear that, to a large extent, they retained their normal form in spite of gross dissolution of white matter (Fig. 30). Where demyelination in the hemispheres was of a mild degree no distinct cell changes were observed. In those cases with gross liquefaction of the white matter, the cortex showed irregularity in arrangement of the ganglion cells, some of which showed shrinkage and pyknosis, others vacuolation and displacement of the nuclei. Sections of the motor area (superior frontal gyrus) were made from many cases. The pyramidal cells were definable but often shrunken and pyknotic, with a clear perineuronal space around them. Similar, but inconstant, changes were observed in many other parts of the system but their significance, if any, is not known. The disorganisation of the nervous system as a whole might have been responsible for many of the changes. For example, small nerve cells were frequently seen deep in the white matter.

In view of the suggestion that the motor tracts of the sheep might (in part) have a subcortical origin, sections of the red nucleus were examined. Unmistakable signs of degeneration were present in the nerve cells of this nucleus in all cases examined. Some showed early chromatolytic changes in the cytoplasm; in others the Nissl granules had completely disappeared with the nucleus shrunken and displaced to the cell margin, the cells having a clear homogenous appearance (Figs. 36, 37).

Neuronphagia was never observed in any part of the system, and it is important to record that after a careful search inclusion bodies could not be found.

Gliosis could be demonstrated in the early demyelinated areas with suitable silver impregnation methods. In those cases with cavity formation the walls were composed of a dense feltwork of glia fibrils (Fig. 32). The cells concerned in this reaction appeared to be the fibrous astrocytes. This gliosis, however, was never so intense as is seen in any of the analogous conditions in man. It is still uncertain whether the oligodendroglia participated at all in the pathological picture. Nevertheless, in grossly degenerated areas there was an impoverishment of all cells apart from the classical astrocytes (Fig. 29). It is probable, therefore, that the oligodendroglia had been destroyed with, or subsequent to, the myelin. Microglial participation was not outstanding either by the formation of rod cells or of the amoeboid macrophagic type (Fig. 29). The paucity of the latter, containing lipoid derived from myelin breakdown, has already been noted.

(3) Identification of the Australian Disease (Enzootic Ataxia) with " Swayback."

The original work by Bennetts (1932) in Western Australia did not include any detailed examination of the brain, and it was uncertain that the disease was identical with " Swayback." Later, in 1937, Bennetts recorded cerebral lesions in Australian cases identical with those described by Innes (1936) for " Swayback."

(Dr. E. Weston Hurst of the Medical Research Institute, Adelaide, South Australia, has also confirmed these findings.*)

Through the courtesy of Mr. H. Bennetts, the brains of four cases of the Australian disease, already fixed in formalin, were received for examination. A brief description of these cases is sufficient to show that the Australian disease is identical with " Swayback."

Case I (*Bennetts, No.* W. 26).—Born unable to stand; death one day later. The brain was soft and flabby; on section there was a symmetrical gelatinous degeneration of the cerebral white matter extending from pole to pole with early cavitation, particularly of the superior convolutions and occipital poles. No lesions were found in other parts of the brain or cord.

Case II (Bennetts, No. W. 37).—Same history as Case I. On coronal section the lesions were of a minimal grade; small patches of cerebral softening with very early cavitation were present mainly at the summits of the superior convolutions. Superficially the brain of this case looked almost normal.

Case III (Bennetts, Lamb No. W. 47).—This animal was first seen when twelve days old with marked ataxia and died five days later. The brain was sodden and flabby; on cross section there was present an identical appearance to that of many cases seen in this country. No normal white matter was present in any part of the cerebrum; demarcation between the grey and white matter was extremely blurred and cavitation was of a minor grade (Fig. 17). The diffuse symmetrical gelatinous nature of the lesion was highly reminiscent of some cases of Schilder's encephalitis seen in children. No lesions in any other part of the brain.

Case IV (Bennetts, Lamb No. 37).—This subject was weak and unthrifty from birth, the condition developed more slowly and the animal died of pneumonia when one month old. The brain, like many others seen in England, showed no macroscopic lesions in any part of the white matter.

A few representative sections from these specimens were examined histologically and it was found that there were no significant differences in the type, nature or distribution of the lesions as compared with " Swayback." Inflammatory changes were absent; demyelination was a constant feature; the cellular lesions in the red nucleus were conspicuous and the cord degeneration was identical.

(4) Pathological Examination of Ewes which have given Birth to " Swayback " Lambs.

It is a notorious fact that ewes, which give birth to " Swayback " lambs, appear to be healthy and may in subsequent seasons give birth to either healthy or affected lambs. There cannot be, therefore, any serious organic disease in these ewes, in spite of the delivery of diseased progeny. Whether this is more apparent than real may be reconsidered later.

Six ewes, which had given birth to " Swayback " lambs a few days previously, were killed and subjected to a *post-mortem* examination.

*Personal communication (1937).

It is unnecessary to give details of the autopsies as in each case significant lesions in any part of the body (including the central nervous system) were absent.

(5) Pathological Examination of "Chronic" or Mild Cases.

Some lambs may develop symptoms a few weeks after birth, and the disease may not progress then beyond a slight but permanent weakness of the hind legs. These animals will survive, and are often used later for breeding.

It was considered that an examination of the nervous system in such cases might be of value. A sheep, one year old, which had shown only such slight non-progressive symptoms after birth was killed. The animal exhibited only a slight weakness of the hind quarters, particularly when made to turn, otherwise the sheep was in a good, healthy condition.

No macroscopic lesions were observed in the viscera nor in any part of the nervous system. A series of sections, stained by Weigert-Pal, were made from the nervous system at regular levels through the cerebrum, mid-brain, pons, medulla and various segments of the spinal cord.

It was expected in such mild cases as this, with a long survival period, that those areas of the white matter which had been demyelinated (presumably sclerosed) would be easily demonstrable by Weigert-Pal technique. This, however, was not the case. The nervous system showed no lesion which was of diagnostic significance, and certainly none recognisable as plaques of demyelination. Transverse sections of the cord stained by Weigert-Pal to all intents and purposes were normal.

It was concluded that, in such mild cases, the number of fibres which undergo degeneration in the hemispheres and in the descending motor tracts of the cord must be so small and/or so scattered as to render their recognition by Weigert-Pal technique impossible.

VI.—Conclusions Regarding the Pathology.

1. Pathological Substratum of "Swayback."—"Swayback" is a nervous disorder of a type which has not been previously described in domestic animals. The lesions which form the anatomic basis of this disorder are essentially those of cerebral demyelination with consequent destruction of the axis cylinders and later cavitation and secondary degeneration of the motor paths. The occurrence of ataxia at birth in most cases, and the presence of severe degenerative lesions in the brains of lambs examined within a few minutes of birth, as well as in stillborn lambs, indicate conclusively that the process is ante-natal in origin. This is not invalidated by the occurrence of cases which do not appear to show symptoms until some time after birth. The symptoms in these animals might be so slight as to evade notice, and in any case, with minimal damage to the nervous

system of the developing lamb late in pregnany, the symptoms may take some time to develop.

2. Demyelination or Myelin Aplasia ?—The lesions might be considered to be due to an inhibition or aplasia of myelin and not to an active destruction of formed myelin. The same problem has arisen in connection with infantile cases of diffuse sclerosis in the human being (*e.g.*, see Bouman (1934), and Russell and Tallerman (1937).) It must be stressed that in every case of " Swayback " the brain as a whole is a well-developed full-time organ in respect of size, conformity and structure. The idea of a myelin aplasia is thus unacceptable, but even more so because it would not explain the porous areas, the gross cavitation, nor the myelin destruction demonstrated by positive staining reactions (Marchi or Scharlach R methods). An active degeneration is also indicated by the changes in the cells of the red nucleus, in the cells of the motor cortex and in the myelin sheaths of the descending motor paths.

It is improbable that the " destruction " of the cerebral white matter can be regarded other than that of an idiopathic disintegration of a substance which had been formed *in utero.* It is, however, feasible that with the onset of demyelination normal myelination would be inhibited.

3. Genesis of the Lesion.—As the condition is primarily an ante-natal demyelination, the state of myelination at the time the causative factor begins to operate must be an influential factor on the subsequent course of the disease. It is, therefore, unlikely that the pathogenesis will be understood until more is known about myelination in the lamb. Simply because the disease is ante-natal in origin the initial stages of the process cannot be studied, and it seems improbable that this will be done until it can be produced experimentally.

It might be suggested however that in " Swayback " the demyelination occurs at a relatively late stage of gestation, perhaps within the last four weeks, *i.e.*, after cerebral myelination has begun.* If this were not so, a greater degree of brain deformity than that seen would almost certainly be effected. The disintegration of white matter must have proceeded occasionally with a singular rapidity, as in some cases seen soon after birth there remained little or no intact cerebral myelin.

The severity and extent of the lesions were not related to the age of the lamb. Some cases between the ages of five and twelve weeks showed no macroscopic changes in the brain ; on the other hand, gross cerebral damage was seen in stillborn lambs and in others a

*Mr. G. J. Romanes, School of Anatomy, Cambridge, has begun an investigation concerning myelination in the lamb. In a personal communication he has informed us that there is little cerebral myelination except in the internal capsules and optic chiasma in a 109 days' fœtus (Fig. 19). On the other hand, the ventro-lateral tracts of the cord appear to begin myelinating about 80 days, and by 124 days are well advanced.

few minutes to four weeks old. While the disease might be thus regarded as a progressive disorder in which cavitation was the end stage of a degenerative process, this was not a constant feature. It is probable that, as well as an acute progressive type, there is also a more chronic non-progressive mild type of the disease, some of the latter cases indeed surviving. The occurrence of these mild cases might indicate that there is an optimal time for the causative agent to operate and that it does so with varied intensity. The damage done in the mild cases is slight, or the cause operates too late in relation to normal myelination to be fully pathogenic, while the " cause " is obviously not continuous beyond the ante-natal period. In support of this latter statement it might be mentioned that it is a common practice to suckle other healthy lambs on ewes which have lost their own lamb or lambs from " Swayback " ; these foster-lambs never exhibit any signs of the disease.

4. Liquefaction and Cavitation in the Cerebral Lesions.—In " Swayback," cavitation was always present when degeneration of the cerebral white matter was intense. This " porencephalic-like " process has its analogy in human pathology, but it must not be identified with true porencephaly due to defective development.

It is recognised that the immature brain of infants reacts to dissolution of tissue by liquefaction and cavity formation, while in the case of the human adult brain glial organisation predominates. The brain of the lamb must be regarded as even more " immature " than the child's brain, and at the age when these lambs are affected glial development and reactivity to a foreign agent or trauma must be of a low grade. In any case, the process appears to be so acute in many lambs that there may not be time for a proper glial functioning. Hence, cavitation might appear to be the only possible reaction to active destruction of white matter in lambs of this age.

The cavitation seen in " Swayback " has also been recorded in Schilder's disease, and diffuse sclerosis in man (*e.g.*, see Bouman, 1934, Russell and Tallerman, 1937).

5. The Ventricular Dilatation in " Swayback."—The internal hydrocephalus of the lateral ventricles which was observed in many cases of " Swayback " has also its parallel in human pathology. The same phenomenon seems to occur in man consequent to any degree of cerebral atrophy, Brain (1933), Wertham (1934), Bouman (1934), Collier and Greenfield (1924), when it has been regarded as a compensatory process for the cerebral atrophy and not due to increased ventricular pressure. In " Swayback," there was also no sign that it was due to any mechanical obstruction, present, for example, in the interventricular foramen, the aqueduct or outlets in the fourth ventricle.

6. The Nature of the Disease.—The nature of demyelinating diseases in man has been discussed so many times, with so much disagreement, that it is fruitless to reopen the question regarding

" Swayback." (Reference can be made to Bouman (1934), Globus and Strauss (1928) and Dawson (1916).) In all papers dealing with this aspect the contentions for and against an inflammatory nature have depended on the criteria used to distinguish inflammation from frank degeneration. It must be emphasised that in " Swayback " (*a*) there never occurs any infiltrations of " inflammatory " cell elements, (*b*) a search for inclusion bodies has been negative and (*c*) the process has an extraordinary affinity for the white matter. These changes are distinct from those found in known infections of the nervous system. " Swayback " is thus regarded as being allied to a true degeneration and not to an inflammatory process.

7. Motor Paths of the Sheep and the Spinal Cord Degeneration in " Swayback."—The motor paths of the sheep have been studied by few workers. King (1911) confirmed a view that the motor area of the sheep was restricted to the superior frontal gyrus. This was ascertained both by stimulation experiments and by histological study, the latter method showing that the Betz cells were confined to this area (see also Bagley, 1922). King showed that the sheep, like many lower animals, possessed a comparatively small pyramidal tract. From hemi-section experiments it was found that descending fasciculi degenerated as a result of a lesion in the motor area, but that most of the fibres decussated in the lower part of the medulla and in the first cervical segment, after which they became scattered and could not be followed further. As a result of a hemi-section lesion in the lower part of the pons and medulla, two other well developed descending paths were demonstrated, (*a*) one in the dorsal part of the ventro-lateral columns of the cord extending to the sacral region, and probably corresponding to the rubro-spinal tract of the cat, and (*b*) a smaller ventral tract in the neighbourhood of the ventral fissure. King concluded that the distinct development of these two tracts might indicate that the principal direct motor paths in the sheep (and other lower animals) are subcortical in origin, and that they might arise from cells in the mid-brain, pons, medulla or even all three regions.

Spinal cord degeneration in " Swayback " was first described by Lyle Stewart (1932) in this country and by Bennetts (1932) in the case of the Australian disease. Both of these workers inferred, however, by analogy with human tracts, that this represented an ascending degeneration. In location and extent, the tracts described by them and those seen constantly in all our own cases of " Swayback," were identical with those described by King. However, little doubt can remain that they are indeed motor in origin and function, as the symptoms are indicative of an upper motor neurone lesion.

The destruction of the cerebral white matter must be regarded as being responsible for pyramidal tract degeneration, but we know that these tracts fade out in the upper part of the cord. If the conclusions of King regarding the origin of the motor tracts of the sheep are correct, then the degenerated tracts in " Swayback " seen in the

upper segments of the spinal cord, represent combined pyramidal and rubro-spinal tracts, while in the lower segments of the cord only the latter.

The suggestion of King that the motor tracts might originate in the mid-brain might have some corollary in this condition of "Swayback." In all cases of "Swayback" examined, acute chromatolytic changes were found in the nerve cells of the red nucleus (Figs. 36 and 37). In any one field taken at random through the nucleus it was common to find six or more cells which had undergone such a change.

The degeneration in the spinal cord, and its relationship to the general pathological picture is, therefore, not yet fully understood. The chromatolytic changes in the cells of the red nucleus may be simply retrograde axonal reaction, yet it is hard to disbelieve that the cord degeneration is not in some way connected with the gross cerebral lesions, in other words, a true secondary descending degeneration. It is possible, of course, that whatever the causal agent may be, it might have an independent action on the nerve cells of the red nucleus as well as the cerebral myelin.

8. Relationship of "Swayback" to Schilder's Disease in Man.—This disease was first described by Schilder (1912) and designated by him as *encephalitis periaxialia diffusa;* since then, by numerous other workers, many of whom have suggested a different name. Good accounts have been written by Collier and Greenfield (1924), Stewart, Greenfield and Blandy (1927), Bouman (1934) and Globus and Strauss (1928), the following being a brief summary taken from these papers.

It usually occurs in infants or children, and is invariably fatal. Clinically, the important triad of symptoms are progressive spastic paralysis, blindness and mental deterioration; the disease is non-febrile throughout its course. Although the majority of the cases appear to be sporadic, an increasing familial incidence has been recognised, *e.g.*, by Symonds (1928), Macnamara and Dickson (1933) and Meyer and Tennent (1936). The outstanding pathological feature is the massive demyelination of the cerebral white matter. According to the stage at which the disease is observed, the lesion may thus affect a part of a lobe, a whole lobe, or even a whole hemisphere, often in a symmetrical manner. The sparing of the subarcuate fibres is frequently stressed, and the lesion may be separated from the cortex by unaffected white matter to form a wide territory parallel with the cortex. The axis cylinders are destroyed at the same or later period. Infiltrations of amoeboid microglia containing broken-down myelin are always present, and infiltration by lymphocytic elements, etc., may be absent, mild, or severe.

This brief description of the disease in man indicates that "Swayback" has many comparable pathological and clinical features. These would include (*a*) the diffuse bilateral demyelination of the cerebrum, often also beginning or being confined to the occipital poles, (*b*) the absence of demyelination in parts of the brain below the cerebral level, (*c*) the relative preservation of the cortical grey matter, (*d*) the blindness in some cases, (*e*) the spastic paralysis and

inco-ordination, (*f*) the progressive course and fatal termination in many cases and (*g*) the present obscure ætiology from which viruses might be definitely excluded.

Too strict an analogy between neuropathological conditions in man and lower animals must not, however be expected, mainly owing to the relative immaturity of the brain of the latter. The disease process in " Swayback " also begins at so early an age that this immaturity might be considered even more pronounced.

As there is a tendency now to regard Schilder's disease only as an anatomical entity, there can be no doubt that " Swayback " is a disease comparable at least to the Schilder's type. Until more is known about the ætiology of " Swayback " and the demyelination diseases in man, it would be unwise to elaborate further.

VII.—Aetiology.

1. Bacteriological and Transmission Work.

A thorough examination was made of different organs, blood, brain, and cerebro-spinal fluid of many cases, using a variety of cultural methods. The cultures of all either remained sterile or showed growths of contaminating bacteria only ; no specific organism was isolated. These findings are in accordance with those obtained in previous years (from 1926 onwards) by other workers in the Institute. Attempts were also made to transmit the disease to healthy lambs. Emulsions of visceral organs or blood or parts of the nervous system from " Swayback " cases were inoculated into healthy lambs by many routes ; all inoculated lambs remained healthy. The view that " Swayback " was not infectious or contagious in nature was supported by the fact that affected lambs or their ewes do not appear to transmit the disease under natural conditions. In an affected area the ewes are usually together throughout pregnancy while " Swayback " lambs are often in contact with young healthy lambs for some time without any indication of transmission of the disease. There is also the fact that the type of lesion in " Swayback " is unlike that which occurs in known transmissible infections of the nervous system. Finally, it is improbable that ewes could carry an " infective agent " for years which would be pathogenic only for the developing lambs of consecutive or, even more puzzling, intermittent pregnancies.

With just reason, therefore, it was concluded that neither bacteria nor viruses were concerned in the ætiology. This conclusion is similar to that of Lyle Stewart (1932) in England, Bennetts (1932) in Australia, Gaiger (1917) in Peru and Magnusson (1920) in Sweden, providing that the condition described by Gaiger and Magnusson was " Swayback."

These negative findings are of importance, as they are parallel to those of many workers who have tried repeatedly, without success, to identify either bacteria or viruses with analogous demyelination diseases in man (*e.g.*, disseminated sclerosis, Schilder's disease,

post-infective and post-vaccinial encephalitis), in monkeys (*e.g.*, the Schilder's-like disease and in dogs (post-distemper encephalitis).

2. Breeding or Hereditary Influences.

There is no evidence to indicate that the disease is either hereditary or developmental in nature. Lambs of most breeds may be affected, and the inter-crossing of ewes and rams of various breeds in consecutive years does not influence the incidence of " Swayback " in the progeny.

It is difficult to believe that the disease is a malformation of development, partly because of its high incidence and wide occurrence, but more particularly because this concept would not explain the singularly rapid degenerative process in the brain.

3. Biochemical Aspects.

Deficiencies of any of the recognised vitamins and lack of the more common minerals might seem on superficial examination to be eliminated.

It might be mentioned that the sheep is able to synthesise vitamins C, D in the presence of sunlight, and some of the B factors in its own body, and as a result they probably suffer no ill-effect from their absence in the food. In many cases of " Swayback," estimations of vitamin A in the livers were made by Dr. T. Moore, Nutritional Laboratory, Cambridge; abundant reserves were always found.

*As a routine measure chemical analyses were made of the blood of a number of cases and of their ewes on a farm in which " Swayback " had been prevalent for some years. The serum calcium, inorganic phosphorus and magnesium were estimated. Most of the values were within the range of normality, but in a few " Swayback " lambs and their mothers a lower serum P was present. Thus, figures such as 7·8 mg., 8·8 mg., 8·1 mg., 8·5 mg., compared with the normal figures of 12 to 13 mg. for the lambs and 3·0 mg. compared with 4 to 5 mg. (per 100 c.c. serum) for the ewes were obtained. The significance of this finding is unknown.

Composition of the Grass from " Affected " and Control Areas.

Grass was collected for chemical analysis free from gross contamination with soil and roots from farms in Derbyshire and Gloucestershire where the disease was prevalent, from farms adjacent to these where the disease was unknown, and from farms in Cambridgeshire far removed from affected areas. No special precautions were observed in collecting the samples as a more accurate assessment of the intake of the various constituents could thus be obtained. The methods outlined in the bulletin issued from the Imperial Bureau of Animal Nutrition (No. 9), were used for the estimation of the commoner constituents. For the estimation of Cu, Pb and Fe a representative sample of the grass was air dried and not ground in a machine but cut into small pieces by clean scissors so that

*These analyses were made by Dr. J. Stewart now of the Animal Diseases Research Association, Moredun Institute, Edinburgh.

contamination from extraneous sources could be eliminated. The method of estimation of Pb in blood, organs and grass was that of Winter *et al.* (1935) and of Cu that of McFarlane (1932), later modified by Tompsett (1934). Digestion was carried out with H_2SO_4, $HClO_4$ and HNO_3, a procedure suggested by Drs. H. H. Green and A. Eden of the Veterinary Laboratories, Ministry of Agriculture, Weybridge. All reagents used were free from Pb and Cu. The results of these analyses are set out in Table I.

With the exception of the Cu, Fe and Pb content of the grass, it will be seen from the table that while the chemical composition of the grass from affected farms compares unfavourably in most instances with that of good pasture, especially with regard to the P_2O_5 content, no gross deficiency of any constituent is evident, and that of the control farms in Derbyshire is similar to that of " affected " farms.

In connection with the Cu, Fe and Pb content of these pastures, the most striking feature is the remarkable constancy of the amount of Cu. Of the 15 samples analysed from widely separated areas, the range is between 1·2 to 2·7 mg. per cent. with no difference between " Swayback " and " control " areas. The Pb content, however, shows a wide range of values. Grass from " Swayback " farms in Derbyshire shows, except in one instance, a Pb content of over 5 mg. per cent., and in one case has the very high value of 39·6 mg. per cent. A high Pb content of grass is also shown in samples from one " Swayback " farm in Gloucester. While grass from control farms in Derbyshire near " Swayback " areas also show similar figures to these, that from farms away from " Swayback " areas contains very small amounts of Pb. The significance of the Cu and Pb figures will be discussed later.

The amount of Fe in the grass from both " Swayback " and control farms in Derbyshire, and also from " Swayback " farms in Gloucester, is much higher than is usually found in grass. The significance of these figures is difficult to assess.

4. Lead Poisoning in Relation to " Swayback."

Lead has for many years been known to produce changes in the nervous system. In chronic lead poisoning of children one of the most dreaded complications is lead encephalopathy. In adults, on the other hand, while cerebral disturbances may occur, the common lesion is a peripheral neuritis affecting the motor nerves. Many reports on the pathology of the nervous lesions have been made, *e.g.*, see Aub *et al.* (1925), Ferraro (1932), Blackman (1937) and Baker (1936). Extreme cerebral œdema may be present and demyelination has been recorded. Putnam (1883) recorded cases of diffuse lesions of the spinal cord and brain with spasticity of the legs in which he found lead in the urine, and as a result suggested the metal as the cause. An association of lead with multiple sclerosis has been suggested since then, *e.g.*, by Eichhorst (1913) and by Cone, Russel and Harwood (1934). It is therefore of interest that a connection between lead and " Swayback " has been considered from entirely different sources (see below) and as yet cannot be dismissed as a possible causal factor.

TABLE I

COMPOSITION OF GRASSES. (On 100 per cent. dry matter).

	Farm.	*Total ash.* *Per cent.*	*Soluble ash.* *Per cent.*	*CaO.* *Per cent.*	P_2O_5 *Per cent.*	*Ether extract.* *Per cent.*	*Crude protein.* *Per cent.*	*Fibre.* *Per cent.*	*Fe.* *Per cent.*	*Cu mg.* *Per cent.*	*Pb mg.* *Per cent.*
A. "Swayback" (Derbyshire) Farms	1	12·02	3·79	0·83	0·64	3·68	17·37	24·31	—	2·4	8·0
	2	13·10	6·89	2·39	0·38	2·92	10·75	27·72	0·0398	1·5	0·9
	3	9·84	3·62	1·04	0·54	3·21	12·44	28·63	0·1326	1·4	7·8
	4	13·80	3·93	0·63	0·47	2·60	12·75	27·01	0·1669	1·4	8·2
	5	14·43	6·95	1·36	0·67	2·54	13·06	28·43	0·1286	2·3	5·2
	6	14·36	5·93	0·93	0·56	3·43	12·37	23·35	—	2·7	39·6
B. Control farms in or near "Swayback" area	1	10·71	4·68	1·3	0·54	4·24	15·38	25·23	0·1432	1·5	6·8
	2	8·54	2·54	0·30	0·49	3·33	13·31	27·42	0·1299	1·9	8·5
	3	10·16	6·53	0·85	0·78	5·49	15·31	17·65	0·0693	1·8	4·4
C. "Swayback" farms (Gloucestershire)	1	9·99	3·42	0·52	0·56	2·63	12·81	25·81	0·1047	1·9	11·9
	2	9·95	4·32	0·72	0·68	3·59	15·94	25·92	0·0880	1·7	1·9
D. Control farm (Northamptonshire		23·33	4·91	2·24	0·74	2·49	16·50	20·27	0·0229	2·4	0·5
E. Control farms away from "Swayback" areas	1	11·80	4·89	0·79	0·91	4·96	14·06	24·13	0·0492	1·2	<0·05
	2	10·12	6·33	0·85	0·66	6·32	22·19	17·85	0·0423	1·4	<0·05
	3	—	—	—	—	—	—	—	0·0467	1·8	<0·05

It was concluded in 1935 that if the disease is caused by some " toxic " substance, it must be carried by the pregnant ewe (over a period of years) to exert a pathogenic effect only on the developing lamb. It was thus essential that this " toxic agent " could pass freely through the placenta in order to affect the brain of the lamb.

These conclusions were similar to those of Bennetts (1935) in Australia who thought that the causal agent might be excreted through the ewe's milk*, and suggested the possibility of a plant or mineral poison and that lead might be the " poison " involved. Analyses of the milk from " affected " ewes showed the presence of traces of lead, although traces were found in the milk of normal ewes. " Deleading " experiments were made by administering ammonium chloride licks to pregnant ewes with an apparent reduction in the incidence of " Swayback " in their progeny. In the absence of other proof, the association of lead with the disease was uncertain.

In the affected area in Derbyshire there are several facts which might also suggest a causal connection of lead with " Swayback." This area is irregularly traversed by what are known locally as " lead rakes "—remains of old abandoned lead mines—which in course of time have been overgrown with grass to which grazing animals have access. Many farmers believe that this " bellanded "† land is the cause of " Swayback " as the name—Belland—given by them to the disease suggests. Instances of " Belland " or lead poisoning in other animals (mainly horses and poultry) are quoted by the farmer to support this belief. (It is necessary, however, to mention that the disease occurs on farms in this and other areas of England where there are no known outcrops of lead). Most of these cases of lead poisoning appear to be sporadic and subacute, and while the animals may suffer from a posterior paralysis, the pathology of the nervous system has not been investigated, so that any comparison with " Swayback " is impossible.

The condition " renguerra " in Peru, which may be similar to " Swayback," has also been associated with lead by Tabusso (see Gaiger, 1917, and Mitchell, 1937).

These various suggestions indicated that lead could not be cursorily dismissed as of no moment in the ætiology, and work was necessary along this line. Chemical analyses were, therefore, made of the pastures from farms in this and other " Swayback " areas and also of various body tissues of " Swayback " lambs and their mothers.

The analyses of the grass from " affected " and " Swayback " areas will be found in Table I ; the extremely high content of Pb is

*It is unlikely that there is any pathogenic " toxic " or " deficiency factor " in the milk, as healthy lambs can be fostered to " Swayback " ewes with no ill effect.

†" Belland," with its variation " Belliam," are terms used in Derbyshire, Yorkshire and Shropshire to indicate the fine dust of lead ore. As this substance is highly poisonous the terms have subsequently meant lead poisoning. In a discussion of lead poisoning in animals, Gardner (1924), mentioned a farm in Yorkshire, apparently notorious for cases in horses, cattle and poultry, in which many of the lambs were " born with rickety backs."

evident. Analyses were made of the livers from cases of "Swayback" and from two ewes which had given birth to " Swayback " lambs. The results are shown in Table IV ; the large amount of Pb in the livers of " Swayback " lambs supports the view that Pb is transported through the placenta. Lead analyses were also made of the brains of 15 cases of " Swayback " ; values were obtained ranging from a trace to 5·5 mg. per 100 grammes dried brain compared with the figures of nil to 0·7 in four control brains.

Conclusions Regarding the Association of Lead with " Swayback."

It is necessary to emphasise certain conclusions of Aub *et al.* (1925). There appears to be only a quantitative difference between the so-called " normal lead " of tissues with that found in cases of poisoning. It is, therefore, essential to determine how much lead is likely to be absorbed by individuals living a normal life at a given time and place. As far as the pathology is concerned, there are few diagnostic lesions apart from the blue line in the gums and the anæmia with stippling of the red cells ; many other anatomical changes have been described, *e.g.*, in the nervous system, but they are not constantly found in every case. There is also some difference in reaction to the metal between children and adults.

As far as lead poisoning in animals is concerned, these facts must be considered and should be investigated because we know little about the animal aspect, especially when it is a matter of deciding whether lead is involved in a condition (" Swayback ") unlike that which has been described in plumbism.

It is known that young animals suffering from lead poisoning show a form of posterior paralysis, not unlike " Swayback," but this parallel symptomology makes it difficult to determine whether Pb is involved in " Swayback." The occurrence of the disease in districts of Derbyshire, Yorkshire and the Mendips, where there is much lead, has perhaps focused too much attention on the metal. Much of our work has been done in Derbyshire and it would be essential to carry out similar investigations in other " Swayback " areas. We know that the disease occurs in parts of England which cannot be compared with Derbyshire as far as the lead content of the soil is concerned, and there may actually occur a lead encephalopathy (" Swayback-like ") in lambs to be distinguished from " Swayback."

We did not find any constant lesion either in ewes or affected lambs which could be regarded as similar to those known in plumbism. The lead line in the gums was occasionally seen in ewes in Derbyshire ; an anæmia with punctate basophilia of the red cells, although present in a number of ewes and lambs, was not a constant feature, and X-ray examination of the bones of one case showed nothing similar to the " lead line " in human lead poisoning. The cerebral lesion in " Swayback " is of no diagnostic help as there are no data regarding the state of the nervous system in animals, particularly young lambs, suffering from lead poisoning. In spite of this, however,

the quantities of lead present in the tissues of " Swayback " lambs (from Derbyshire and other areas, Table IV) and of the ewes must be highly abnormal, while the ewes must ingest during grazing relatively large amounts of lead over a prolonged period, although in what form or with what significance we do not know.

If lead is connected causally with " Swayback " it must be absorbed insidiously by the ewes so that they do not suffer from outspoken plumbism, as we know can happen in the human being. In that light, feeding experiments might not be successful in proving or disproving any possible causal connection. An initial experiment was, however, carried out with two sheep (one pregnant) for this purpose. The dose of lead acetate given, small though it appeared, (0·5 grammes *per diem*), was sufficient to cause death from lead poisoning in 45 days. Chemical analyses were made to determine the distribution of the metal in various tissues of these animals. The results are shown in Table II. (One important fact emerged from these analyses, namely, that in the pregnant sheep, like in the rat (Bauman, 1933) and in women (Boyd, 1938), the metal can pass through the placenta to the fœtus. In Japan congenital plumbism has been ascribed to the pregnant mother having used cosmetic powders containing lead (see Boyd, 1938).)

Table II

Distribution of Lead in Organs of Sheep Dosed with Lead Acetate

Total weight in mg. of Pb in each organ.

	Sheep I.	*Sheep II.*
Spleen	0·63	1·32
Liver	17·06	10·46
Kidneys (both) ...	10·98	24·28
Brain	0·32	1·17
Foetus ...	—	0·96

These facts suggest that until we know more about the exact ætiology of " Swayback," lead cannot be ignored as a possible causal factor. It need not be, and in fact cannot be, direct plumbism, but we do not know, for example, how lead might affect the assimilation of other substances. As Minot (1938) has stated, there can be no doubt that the continual absorption of lead in quantities, which might still be regarded as relatively small, results in " poisoning." The urgent need at the moment, therefore, is to establish a more exact set of criteria which would enable us to recognise the early ætiological connection between lead and injuries to the animal organism.

5. Anæmia and Copper Deficiency in Relationship to " Swayback."

Bennetts and Chapman (1937) gave a fresh stimulus to investigations along this line when they contended that the disease in Australia

was due to a copper deficiency. Their observations are summarised as follows : Pregnant ewes pastured on " affected land " developed a macrocytic hyperchromic anæmia and as a result their lambs were affected with " Swayback." The copper content of the blood, liver and milk of these anæmic pregnant ewes was much below normal. The feeding of copper to the ewes caused a marked response in the blood picture with a reticulocyte crisis, and the anæmia was thus prevented or cured ; as a result the progeny were not affected with " Swayback." This anæmia of pregnancy was considered as similar to pernicious anæmia of man although it did not respond to liver therapy in the few cases tried. Copper sulphate was claimed to arrest the progress of the disease in the lamb although the initial ataxia was not improved. (In England many mild cases do not progress and may survive without any therapeutic treatment). A few analyses showed that there was no copper deficiency present in the grass compared with that from affected farms, and it was contended that either, the copper content of affected pastures varied throughout the year or, that there was always an adequate amount present, but that some unknown factor interfered with its proper assimilation. These findings await further confirmation, but in any case, the pathogenesis requires explanation. The results of prophylactic copper treatment carried out on a small scale by Bennetts in Australia were, however, remarkable and, if confirmed, must lead to further important discoveries. (A field trial was subsequently carried out in England by Dunlop and Wells (1938) to determine the prophylactic effect of trace elements on the incidence of " Swayback." The results supported the Australian work.) A relation between copper and erythropoiesis has been long contended, but that such a trace element might be vitally connected with myelin formation is altogether a new observation, and may suggest another approach to problems of certain nervous disorders. Further, the occurrence of a macrocytic hyperchromic anæmia has not been established in animals, and if an association of an anæmia of this type with a demyelination disorder (" Swayback ") is confirmed, the problem again offers attractive possibilities from the aspect of comparative pathology.

(*a*) *Recent work on the physiological role of copper in the animal organism.*

While there is evidence to show that copper is essential for normal erythropoiesis, the exact rôle of the metal is not fully understood, and many of the reports in the literature are highly conflicting. Much information is to be obtained in the papers of Hart, Steenbock, Waddell and Elvejhem (1928 and 1929), McHargue, Heally and Hill (1928), Morison and Nash (1930), Beard and Myers (1931), Cunningham (1931), Parsons (1933), Mackay (1933), Stein and Lewis (1933), Sachs, Levine and Fabian (1935), and Tompsett and Anderson (1935). The whole position was reviewed by Elvejhem (1935). The work of these investigators in general seems to indicate that, in the presence of iron, copper stimulates the regeneration of hæmoglobin in animals made anæmic by a milk diet. In

addition, copper alone appears to catalyse the formation of red cells, as animals receiving copper without iron show a rise in the erythrocyte count although a low haemoglobin persists. In experimental milk anæmia there is a specific deficiency of copper which must be corrected, before the utilisation of iron, for the production of hæmoglobin and before the formation of erythrocytes. In the case of spontaneous anæmias of man, the position is not so clear and can be summed up in the words of Janet Vaughan (1936) who stated "that copper probably plays some part in hæmatopoiesis, possibly by assisting in the conversion of inorganic iron into hæmoglobin, thus speeding on the process of red cell maturation. At present we can recognise no anæmia due specifically to lack of copper and no anæmia cured by treatment with copper only." In the case of domestic animals there have been suggestions that copper might be responsible for certain diseased conditions. For example, Neal, Becker and Shealy (1931) observed a recurring nutritional anæmia in cattle in Florida due to a deficiency of Fe and Cu in the vegetation, which could be cured by supplements of Fe and Cu but not by Fe alone. Thomson and Wheeler (1932) produced a nutritional anæmia in lambs by a diet of cow's milk; only a slight response was obtained by feeding $FeCl_3$ free from Cu, but when both $FeCl_3$ and $CuSO_4$ was given, a rapid return of the hæmoglobin to normal levels was obtained. Similarly, Sjollema (1933 and 1938) recorded that in certain districts of Holland the vegetation was deficient in Cu; "lecksucht," a disease of cattle, in which anæmia was a feature, was prevalent in these areas and this condition was cured by copper sulphate treatment. The immediate effect of copper treatment was, however, an apparent increased food consumption, the rise in the hæmoglobin level occurring later, and Sjollema suggested that the action of the copper was primarily on appetite and not on blood formation.

Investigations were necessary along the lines indicated by the above observations; this involved much hæmatological and chemical work which is now dealt with separately.

(*b*) *Hæmatology.*

The purpose of this aspect of work was to examine the blood of a large number of pregnant ewes in farms in the Derbyshire area where "Swayback" was prevalent. The suggestion of Bennetts (1937) that a macrocytic hyperchromic anæmia occurred in ewes which gave birth to "Swayback" lambs, could only be investigated in this way.

About one month before lambing blood examinations were made of many pregnant ewes on different farms in this area. The same ewes were examined again after lambing and to determine whether healthy or "Swayback" lambs had been born. Blood examinations of many "Swayback" lambs and their mothers were also made in the same district. Although several hæmatological studies of sheep blood have been made by previous workers (*e.g.*, see Fraser, 1929), blood examinations were also made of a number of normal ewes and lambs in different parts of the country as controls. This was necessary to obtain hæmatological data which was not available in the literature and in order to correlate the blood picture with the Cu blood values.

Hæmatological examinations were thus made of the following groups of animals : (1) Normal pregnant ewes in non-affected areas, *e.g.*, in Cambridgeshire and Leicestershire ; (2) Normal pregnant ewes from a farm in Derbyshire near a " Swayback " area but on which the disease did not occur ; (3) Pregnant ewes from " Swayback " farms which subsequently gave birth to normal lambs ; (4) Pregnant ewes from " Swayback " farms which gave birth to " Swayback " lambs ; (5) Normal lambs and young sheep in " non-affected and affected " areas ; (6) " Swayback" lambs.

Technique.—All blood examinations were carried out on venous blood obtained either from an ear vein or from the jugular vein. Hæmoglobin estimations were made with a Hellige hæmoglobinometer standardised against O_2 capacity measurements; red and white cell counts were made in the usual routine manner, smears were stained by the May-Grunwald Giemsa method for examination of the red cells and differential leucocyte counts. In many cases observations were also made on the sedimentation rate, reticulocyte count, percentage volume of packed cells, colour index and Price-Jones curves. There was no information available regarding red cell diameters of domestic animals; we have, therefore, prepared a red cell diameter distribution curve for a normal ewe.

After the work had been in progress for some time it was obvious that much of the hæmatological data was irrevelant as there were no significant deviations from normal standards. In the following chapters therefore only those data are given which may have some bearing on the ætiology of " Swayback " as suggested by Bennetts and Chapman (1937).

Normal Standards of Sheep Blood.

From our own examinations the following have been accepted as normal values (Table III).

Table III

Erythrocyte count (millions per c.mm.)	6–11
Hæmoglobin per cent.	60–80
Packed cells (c.c. per 100 c.c. of blood)	26–38
Colour index	0·5–1
Mean corpuscular volume (cubic microns)	34–50
Mean corpuscular hæmoglobin (micromicrogrammes) ...	12–20
Diameter of erythrocytes (microns)	3–6
Reticulocytes (per cent.)	0–0·1
Leucocytes (thousands per c.mm.)	5–14

A large series of hæmatological examinations, such as have been made in man, have not been done on the normal sheep. Before discussing the hæmatological findings in " Swayback " certain facts concerning sheep hæmatology must be emphasised. The above standards are very arbitrary as deviations were found to occur in apparently normal animals within even wider limits than those given, with the exception perhaps of the red cell diameters. In our relatively small series it is evident that the age of sheep is responsible for extraordinary variations (see also Fraser, 1929). For example,

	Case No.	Lambs. Hb Per cent.	RBC ×10⁶	Cell Vol. Per cent.	Cu in blood mg. Per cent.	Cu in liver mg. per cent. Dry Wt.	Pb in liver mg. per cent. Dry Wt.	Mothers of Lambs. Hb Per cent.	RBC ×10⁶	Cell Vol. Per cent.	Cu in blood mg. Per cent.	Cu in liver mg. per cent. Dry Wt.	Pb in liver mg. per cent. Dry Wt.	Grass. Cu mg. Per cent.	Pb mg. Per cent.
	1	74	8·4	43·5	0·065	0·78	0·56	58	8·3	29·7	0·050	—	—	—	5·22
	2	75	8·0	40·5	0·049	0·79	0·34	47	6·4	22·2	0·059	—	—	1·82	25·84
	3	66	8·8	35·5	0·056	0·46	0·40	40	5·0	21·2	0·037	—	—	—	—
	4	82	8·9	27·4	0·062	1·36	—	—	—	—	—	—	—	2·4	8·01
	5	—	—	—	—	1·11	1·57	—	—	—	—	—	—	1·82	25·84
	6	63	6·9	29·6	0·042	0·52	2·51	34	5·1	18·5	0·039	0·68	0·33	—	—
Twins	7	74	7·3	32·2	0·039	1·12	1·29	65	5·8	—	—	1·75	0·44	1·82	25·84
Twins	8	75	6·6	33·7	0·050	0·89	—	—	—	—	—	—	—	—	—
	9	69	6·9	—	0·039	1·10	1·08	62	7·7	28·8	0·058	—	—	1·70	4·11
	10	77	9·2	—	—	0·95	0·27	43	6·0	25·0	0·039	—	—	1·73	5·32
	11	60	10·0	29·0	0·072	0·85	1·63	60	8·6	24·9	0·084	—	—	2·17	6·90
	12	48	7·7	—	—	—	—	54	7·5	26·0	0·070	—	—	2·17	6·90
	13	61	9·0	26·8	0·064	0·89	0·39	41	6·2	22·8	0·060	—	—	1·82	25·84
	14	59	7·8	27·8	0·070	0·95	—	48	6·2	24·2	0·078	—	—	1·82	25·84
	15	68	9·3	38·3	0·066	1·55	0·25	43	6·5	21·8	0·068	—	—	1·82	25·84
	16	79	8·6	33·8	0·050	1·14	0·60	40	6·1	17·9	0·056	—	—	1·82	25·84
	17	69	7·0	30·3	0·044	—	—	56	5·8	26·6	0·060	—	—	1·70	4·11
Twins	18	94	10·9	47·0	0·084	—	—	56	6·4	—	0·048	—	—	1·67	4·72
Twins	19	72	8·0	22·2	0·061	—	—	—	—	—	—	—	—	—	—
	20	—	—	—	0·065	—	0·26	—	—	—	—	—	—	1·82	25·84
	21	92	11·0	46·0	0·048	—	—	—	—	—	—	—	—	—	—
	22	70	8·8	—	—	—	—	—	—	—	—	—	—	—	—
	23	90	9·9	44·5	0·060	—	—	—	—	—	—	—	—	—	—
	24	60	7·8	—	—	—	—	—	—	—	—	—	—	—	—
	25	62	7·9	39·0	0·049	—	—	—	—	—	—	—	—	—	—
	26	62	6·9	—	0·024	—	—	70	7·3	27·4	0·062	—	—	—	—
	27	68	7·5	38·4	0·047	—	—	—	—	—	—	—	—	—	—
Aver.:		70	8·4	35·0	0·055	0·96		51	6·6	24·1	0·058				
								B. GLOUCESTERSHIRE							
		—	—	—	—	—	—	64	7·4	33·7	0·103	—	—		
		—	—	—	—	—	—	72	12·9	36·5	0·077	—	—		
		—	—	—	—	—	—	72	7·6	29·2	0·084	—	—	1·9	11·9
		—	—	—	—	—	—	76	11·9	36·0	0·080	—	—		
		—	—	—	—	—	—	42	6·7	25·2	0·069	—	—		
		—	—	—	—	—	—	72	7·2	35·6	0·047	—	—		
		—	—	—	—	—	—	66	6·6	29·9	0·046	—	—	1·7	1·9
		—	—	—	—	—	—	68	6·5	29·5	0·048	—	—		
							Average	66	8·4	31·9	0·069				

TABLE V

HAEMATOLOGICAL FINDINGS AND Cu ANALYSES OF BLOOD OF EWES FROM "SWAYBACK" AND NON-"SWAYBACK" AREAS GIVING BIRTH TO NORMAL AND "SWAYBACK" LAMBS AND OF NORMAL AND "SWAYBACK" LAMBS.

A. Normal Pregnant Ewes from Non-affected Areas.

CAMBRIDGESHIRE.

Ewe No.	*Hb Per cent.*	*RBC* $\times 10^6$	*Cell Vol.*	*Cu mg. Per cent.*
1	83	9·0	30·5	0·136
2	75	8·7	30·5	0·124
3	73	8·2	33·7	0·128
4	77	8·3	37·4	0·115
5	80	8·2	—	0·130
6	70	8·0	29·8	0·125
7	74	9·1	34·4	0·111
8	71	9·6	38·9	0·128
9	72	9·6	37·5	0·138
10	76	8·1	38·8	0·125
11	67	8·7	31·5	—
12	70	8·4	33·8	0·133
13	62	6·8	33·4	0·160
14	60	7·6	29·6	0·281
15	60	6·6	27·9	0·130
16	64	9·3	33·9	0·089
17	70	8·6	38·0	0·054
18	72	10·0	—	0·055
Aver.:	71	8·5	33·7	0·127

LEICESTERSHIRE.

Ewe No.	*Hb Per cent.*	*RBC* $\times 10^6$	*Cell Vol.*	*Cu mg. Per cent.*
1	75	6·6	36·0	0·050
2	76	6·0	33·3	0·127
3	70	6·4	33·6	0·093
4	75	5·4	38·3	0·051
5	71	6·0	32·6	0·047
Aver.:	73	6·1	34·8	0·073

Non-"Swayback" Area (DERBY).

Ewe No.	*Hb Per cent.*	*RBC* $\times 10^6$	*Cell Vol.*	*Cu mg. Per cent.*
1	65	5·9	29·1	0·082
2	65	6·6	30·7	0·092
3	65	5·2	37·8	0·086
4	65	5·9	28·1	0·122
Aver.:	65	5·9	31·4	0·095

B.—Ewes on "Swayback" Farms giving Birth to Normal Lambs.

Ewe No.	*Hb Per cent.*	*RBC* $\times 10^6$	*Ewe No.*	*Hb Per cent.*	*RBC* $\times 10^6$	*Ewe No.*	*Hb Per cent.*	*RBC* $\times 10^6$	*Cell Vol.*	*Cu mg. Per cent.*
1	60	6·0	21	50	4·5	40	50	4·2	27·8	0·040
2	70	5·0	22	66	5·3	41	50	4·5	—	0·040
3	65	—	23	58	4·5	42	55	4·8	33·2	0·036
4	62	—	24	56	4·0	43	55	4·7	31·7	0·037
5	73	4·0	25	55	4·2	44	60	4·9	26·7	0·034
6	60	3·0	26	54	3·0	45	53	3·5	26·1	0·039
7	50	2·0	27	57	4·2	46	53	3·7	26·5	0·041
8	58	6·0	28	58	3·0	47	44	3·8	—	0·054
9	50	3·0	29	50	3·0	48	53	5·9	28·2	0·050
10	62	—	30	44	3·5	49	69	7·7	44·9	0·049
11	55	6·6	31	60	4·0	50	56	4·6	—	0·045
12	50	3·1	32	60	4·0	51	70	5·6	33·9	0·054
13	47	5·2	33	65	5·0	53	57	5·6	29·7	0·061
14	62	3·0	34	75	5·0	54	65	5·8	—	0·044
15	47	3·0	35	65	5·2	55	54	4·9	—	—
16	60	3·0	36	70	5·4	56	49	4·7	—	—
17	50	3·0	37	67	5·0	57	61	5·0	—	—
18	56	4·0	38	66	5·2	58	60	5·2	—	—
19	50	3·0	39	69	5·0	59	64	5·2	—	—
20	75	—				60	46	4·9	—	—
						61	53	5·8	—	—
						62	64	5·2	—	—
						63	60	4·8	—	—
						66	51	3·0	39·7	0·037
						67	70	4·0	32·6	0·034
						68	70	5·5	—	0·045
						69	62	5·3	30·9	0·048
						70	85	9·4	35·2	0·047
						Aver.:	59	4·9	31·9	0·045

TABLE V—(*Continued*)

C.—Control Ewes (1 *year old*). (CAMBRIDGESHIRE.)

Ewe No.	*Hb Per cent.*	*RBC* $\times 10^6$	*Cell Vol. Per cent.*	*Cu mg. Per cent.*	*Ewe No.*	*Hb Per cent.*	*RBC* $\times 10^6$	*Cell Vol. Per cent.*	*Cu mg. Per cent.*
1	71	11·54	31	0·153	13	77	10·88	30	0·155
2	69	8·31	27	0·138	14	80	11·74	31	0·139
3	85	10·13	31	0·180	15	86	12·39	29	0·157
4	75	12·34	25	0·148	16	92	13·32	32	0·164
5	88	12·02	32	0·143	17	84	11·51	26	0·123
6	77	11·25	35	0·151	18	80	10·45	31	0·137
7	88	10·13	33	0·128	19	80	12·18	26	0·145
8	83	11·98	30	0·153	20	85	12·69	31	0·134
9	75	12·00	37	0·133	21	77	10·36	36	0·157
10	85	9·89	33	0·130	22	72	10·93	32	0·147
11	84	12·73	34	0·143	23	88	11·76	34	0·133
12	77	9·68	31	0·161					
					Aver.:	81	11·09	31	0·146

D.—Normal Lambs.

Non-affected areas (CAMBS.)					Affected area (DERBY).				
Ewe No.	*Hb Per cent.*	*RBC* $\times 10^6$	*Cell Vol.*	*Cu mg. Per cent.*	*Ewe No.*	*Hb Per cent.*	*RBC* $\times 10^6$	*Cell Vol.*	*Cu mg. Per cent.*
1	64	9·0	32·4	0·061	1	75	7·2	—	—
2	67	7·5	—	0·075	2	85	7·9	—	—
3	65	10·9	—	0·379	3	85	8·5	—	—
4	67	12·2	34·2	0·057	4	74	7·9	—	—
5	65	12·2	36·4	0·180					
6	70	13·7	32·3	0·200	Aver.:	80	7·9		
7	70	10·7	—	0·232					
8	74	16·2	36·6	0·232					
Aver.:	68	11·5	34·4	0·177					

in new-born and young lambs there occurs normally an exaggerated physiological polycythæmia and the red cells may number 13·0 to 16·0 millions per c.mm. (Table V, D). With advancing age the red cells appear to decrease in number, although in a number of normal year-old non-pregnant ewes the red cell counts were still much higher than in the many older pregnant and non-pregnant ewes which were examined (Table V, A and C). Apart from this age variation, the hæmoglobin and red cells might be affected by locality, *e.g.*, compare our findings in ewes in Cambridgeshire, Leicestershire and Derbyshire (Table V, A). Recent work by Barcroft, Kennedy and Mason (1939) has also shown that during pregnancy in the sheep there is a rise in blood volume with a decrease in the hæmatocrit

reading. There are thus many factors to be considered when assessing the significance of hæmatological findings in sheep, while an opinion on the blood of any one sheep without knowing more of the influence of age, locality and of pregnancy becomes virtually impossible.

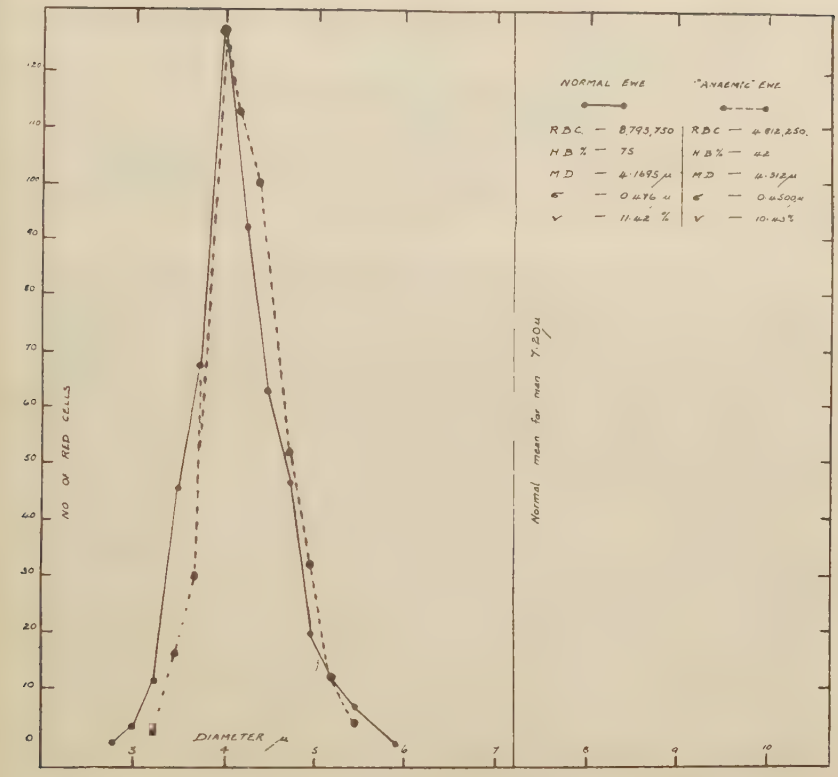
It is beyond the scope of this paper to deal with such matters, but we have sufficient data to show that, as far as " Swayback " is concerned, there is no uniformity in the blood picture of ewes giving birth to " Swayback " lambs ; the findings of Bennetts and Chapman (1937) have, therefore, not been confirmed. (They stated that ewes giving birth to " Swayback " lambs suffered from a macrocytic hyperchromic anæmia comparable with pernicious anæmia in man.)

Blood of Ewes in " Swayback " Areas.

The relevant hæmatological data in the ewes are given in Tables IV, V, A and B. The uniformly lower level in the hæmoglobin and red cells of pregnant ewes in a " Swayback " area in Derbyshire (Table V, B) compared with control ewes in Cambridgeshire, (Table V, A) might indicate the occurrence of a specific anæmia. There is, however, no correlation between this anæmia in the ewe and the occurrence of " Swayback " in the progeny. Ewes in this area giving birth to healthy lambs may show a similar low hæmoglobin and red cell count to those giving birth to " Swayback " lambs (compare Tables IV, V, B). Further, many ewes, both in Derbyshire and in another area in Gloucestershire, which gave birth to " Swayback " lambs, showed a blood picture which must be regarded as within the normal limits (Table IV). (It must be repeated that these latter ewes never seem to show any sign of ill health and it is thus difficult to consider that there could exist in these ewes an anæmia of a type which would be expected to produce grave symptoms.)

Any attempt to substantiate this " anæmia " of pregnant ewes in Derbyshire is beset with difficulties which have already been mentioned. In some of these ewes there may be a greater reduction of red cells than hæmoglobin, thus giving a high colour index, but this is not constant ; and a determination of red cell diameter curves (Price-Jones) of many of these cases showed no significant differences from those obtained in a series of normal ewes (see fig.). Blood films from some of these ewes showed a marked degree of anisocytosis, poikilocytosis, and punctate basophilia, but this again was not constant, and in many animals with a low hæmoglobin and red cell count the erythrocytes appeared normal.

We have thus no evidence to show that a macrocytic hyperchromic anæmia occurs in the mothers of " Swayback " lambs. Macrocytosis is undoubtedly absent, and we have no evidence that the anæmia is even hyperchromic. Barcroft's observation of a raised blood volume and decreased hæmatocrit during pregnancy might indicate that the " anæmia " could be explained partly on this physiological basis and that the lowered hæmoglobin and red cells might not indicate the existence of anæmia at all. Nevertheless it must be



Red cell diameter distribution curve for a normal pregnant ewe compared with one for an "anæmic" ewe.

remembered in any consideration of pregnancy anæmias in animals that a macrocytic hyperchromic anæmia of pregnancy which is not pernicious anæmia occurs in women.

Hæmatology of the "Swayback" Lambs.

There is no uniformity either in the hæmoglobin or red cell counts (Table IV). Many of the very severe cases showed values within the normal range while other cases showed a reduction in both hæmoglobin and red cells. In this connection it must be stated that if the acute cases live for any length of time they undoubtedly suffer from malnutrition; some of them require to be artificially fed while others have great difficulty in obtaining enough milk from the ewes.

(*c*) *Chemical Work.*

Analytical Technique.—For the estimation of Cu in whole blood, the blood sample was taken from the jugular vein into bottles washed with glass distilled water and in which 1 ml. of 10 per cent. sodium oxalate solution free from Cu had been dried. A few drops of blood were allowed

to flow from the bleeding needle before collection to prevent any contamination with impurities from the needle.

The Copper Content of the Blood of Pregnant Ewes from Normal and " Swayback " Areas.

For this purpose blood was taken from most of the sheep, from normal and " Swayback " areas, used for the establishment of hæmatological standards and are tabulated in the same tables (IV and V).

The average figure of 0·127 mg./100 ml. whole blood obtained from normal control pregnant ewes (about one month before lambing) in Cambridge (Table V, A) is a somewhat lower value than that of 0·172 mg. given by Tompsett (1934) for normal sheep. Both figures are, however, much higher than that of 0·075 mg. given by Bennetts and Chapman (1937). The latter value is, however, similar to our figures obtained from ewes on control farms in Derbyshire which were adjacent to " Swayback " areas (Table V, A). Pregnant ewes from " Swayback " farms in Derbyshire giving birth to normal lambs (Table V, B) showed an average of 0·045 mg. per cent. of Cu in the blood which is much lower than that of 0·095 mg. per cent. from ewes in adjacent farms in which the disease is unknown (Table V, A). This value for blood Cu from ewes on " Swayback " farms giving birth to normal lambs was similar to that of 0·058 mg. per cent. (Table IV) from ewes which gave birth to " Swayback " lambs.

The Copper Content of the Blood of Young Normal Sheep and of " Swayback " Lambs.

As no figures for the Cu content of the blood of normal young lambs were available, samples were taken from eight lambs between two and four weeks old and the Cu estimated (see Table V, D).

Although the number of analyses is small, it is evident that young lambs contain a much higher concentration of Cu in the blood than mature sheep. This is in accordance with work done in other species ; for example, Cunningham (1931) found that the Cu content of the liver of fœtal and new-born rats was much higher than that in adult rats. Similar findings have been recorded by Morison and Nash (1930) in the human being.

Cu analyses of the blood and liver from cases of " Swayback " and from their mothers were made, and the results are given in Table IV.

It will be seen from Tables IV and V, D, that the Cu content of the blood of " Swayback " lambs is much lower than that of normal lambs in non-affected areas, although the average figure is higher than that given for the blood of " Swayback " lambs by Bennetts and Chapman (1937) ; the Cu content of the mother's blood is likewise low, and although not constant, there seems to be a relationship between the blood Cu of the lamb and its mother.

The Cu in the livers of these " Swayback " lambs (Table IV) is of the same order of magnitude as that found by Bennetts and Chapman (1937) and is much lower than the normal figure of 14·2 mg./100 grammes dry liver given by Young (1937).

Cu analyses of the livers from two ewes which had given birth to " Swayback " lambs also showed a low figure compared with normal values given by Cunningham (1931).

Conclusion from the Chemical Findings.

These results support our belief that a more extensive investigation is required along the lines indicated above. For example, it would be profitable to follow up the changes in the blood (hæmatological and chemical) of a large number of ewes (during pregnancy and on an affected area) some of which were given copper supplements and others to remain untreated and serve as controls. At the time this paper goes to press a large-scale field experiment is in progress* to determine more exactly the prophylactic effect of copper and to provide an opportunity for work outlined above.

In the meantime, it is suggested that our results indicate that " Swayback " might be another example of a disease due to a trace element, *i.e.*, to a copper deficiency, although the mode of action of this metal in the production of the disease is unknown. It might be a dangerous hazard to speculate at this stage, but the following conclusions based on our chemical findings and on the fact that Cu supplements have had a beneficial prophylactic effect on the disease in Australia and in this country, are worthy of consideration.

(1) A depletion of Cu reserves in the ewe takes place before the birth of an affected lamb ; this is reflected in a reduction in the Cu stores of the newly-born " Swayback " lamb compared with a normal animal in Cambridge (see Table IV).

(2) The level of the Cu in the blood of the mother is not the primary factor which determines the development of " Swayback " in the offspring (compare the figures in Tables IV, V, B).

(3) On the assumption that the Cu reserves in " Swayback " lambs and their mothers are below normal, but that the pastures are not deficient in Cu compared with suitable controls (Table I), the following circumstances might account for the Cu " deficiency " :

(*a*) The Cu is present in the grass in such a form that the ewes are unable to absorb it from the alimentary tract ;

(*b*) There is some other factor present in the grass which inhibits the absorption of Cu ;

(*c*) There exists some dysfunction of the alimentary tract which inhibits or limits the absorption of Cu from the grass ;

*In collaboration with Dr. G. Dunlop, Derbyshire County Council, and Mr. H. E. Wells, Agricultural Organiser, N.W. Derbyshire.

(*d*) The Cu is absorbed in adequate amounts but the rate of elimination is higher than in normal animals.

In the meantime further discussion is profitless until the experimental work at present in progress is terminated. We believe that there is sufficient evidence to indicate that the ætiology may be solved along these lines.

VIII.—Summary.

1. " Swayback " is a nervous disorder of new-born and young lambs of different breeds occurring in many parts of England, Scotland and Wales. The same disease occurs in Australia and New Zealand and probably corresponds to conditions which have occurred in South America, Sweden, South Africa and India. The incidence in Britain varies annually and may be as high as 90 per cent. of the lambs born on any one affected farm. In some areas (*e.g.*, in Derbyshire) the disease is enzootic.

2. The symptoms are those of a spastic paralysis of the limbs with-resultant inco-ordination and occasionally blindness ; in most cases the disease is progressive with a fatal termination.

3. The pathology is characterised by a diffuse symmetrical demyelination of the cerebrum, varying in extent in different cases from small foci in the centrum ovale to gross demyelination of the whole hemispheres. Liquefaction and cavitation is a common end-stage of the lesion. Secondary degeneration of the motor tracts in the cord is always present. The disease is a degenerative disorder bearing some resemblance to Schilder's disease in man and is of ante-natal origin.

4. Bacteria and/or viruses are not concerned in the ætiology ; " Swayback " is analogous in this respect to the demyelinating disorders in man, monkey and the dog.

5. The causal agent causes no obvious disturbance in the health of the ewe but exerts a pathogenic effect on the fœtus or young lamb. This agent has a specific affinity for the cerebral myelin and/or for the mechanism or cells responsible for the laying down of myelin which it destroys with singular rapidity.

6. The suggestion that a disturbance of copper metabolism in the pregnant ewe is concerned in this way with the ætiology has been investigated. Chemical analyses of the blood of " Swayback " lambs and of their mothers show lower Cu values compared with suitable controls. The success which has attended the prevention of " Swayback " by feeding copper to the pregnant ewes in Australia and England indicate that this view might be confirmed. The exact role which copper plays in the ætiology is not, however, understood, as it is apparent from the Cu analyses of affected pastures that the disease is not a Cu deficiency *per se*. The suggestion that Cu deficiency causes a macrocytic hyperchromic anæmia in pregnant ewes and that

as a result the progeny are affected with " Swayback " has not been confirmed.

7. At present we cannot exclude the possibility that Pb is concerned in some way with the development of the disease.

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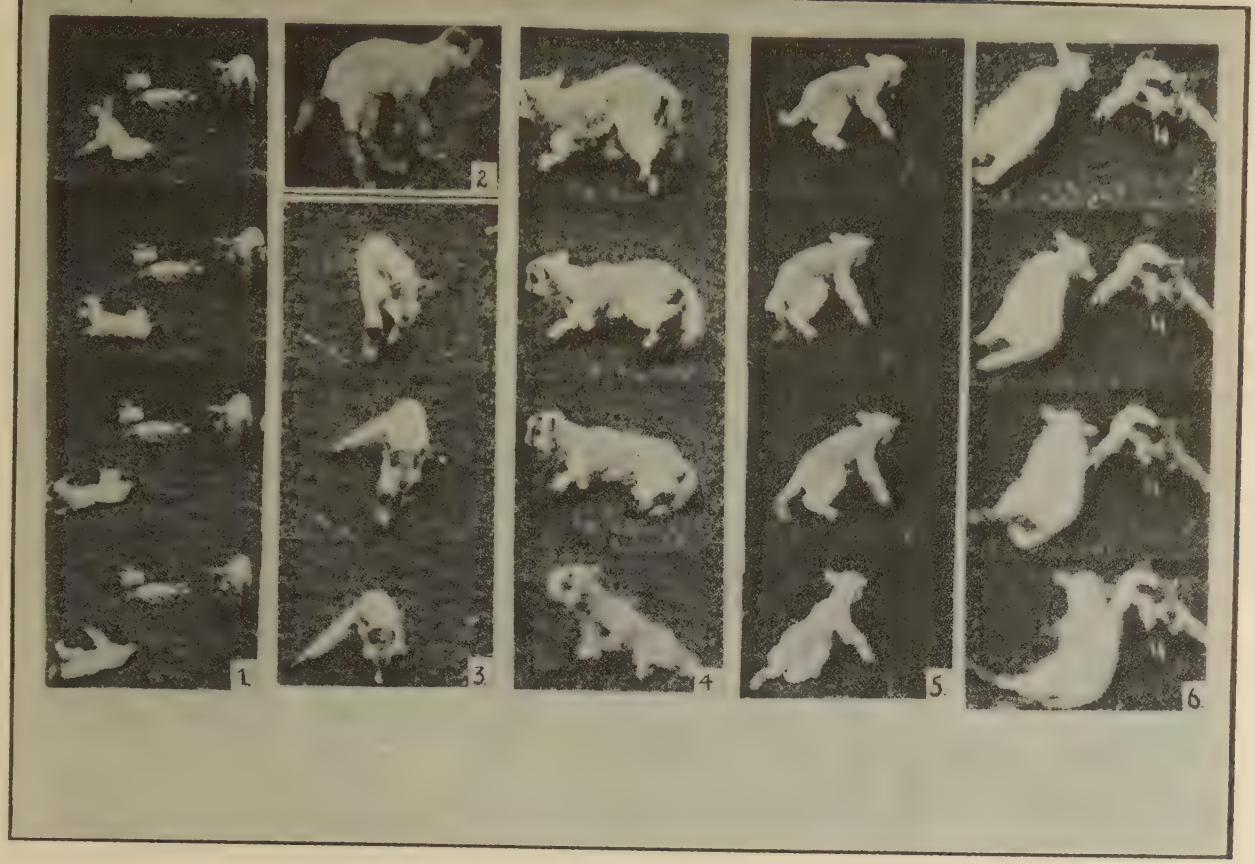
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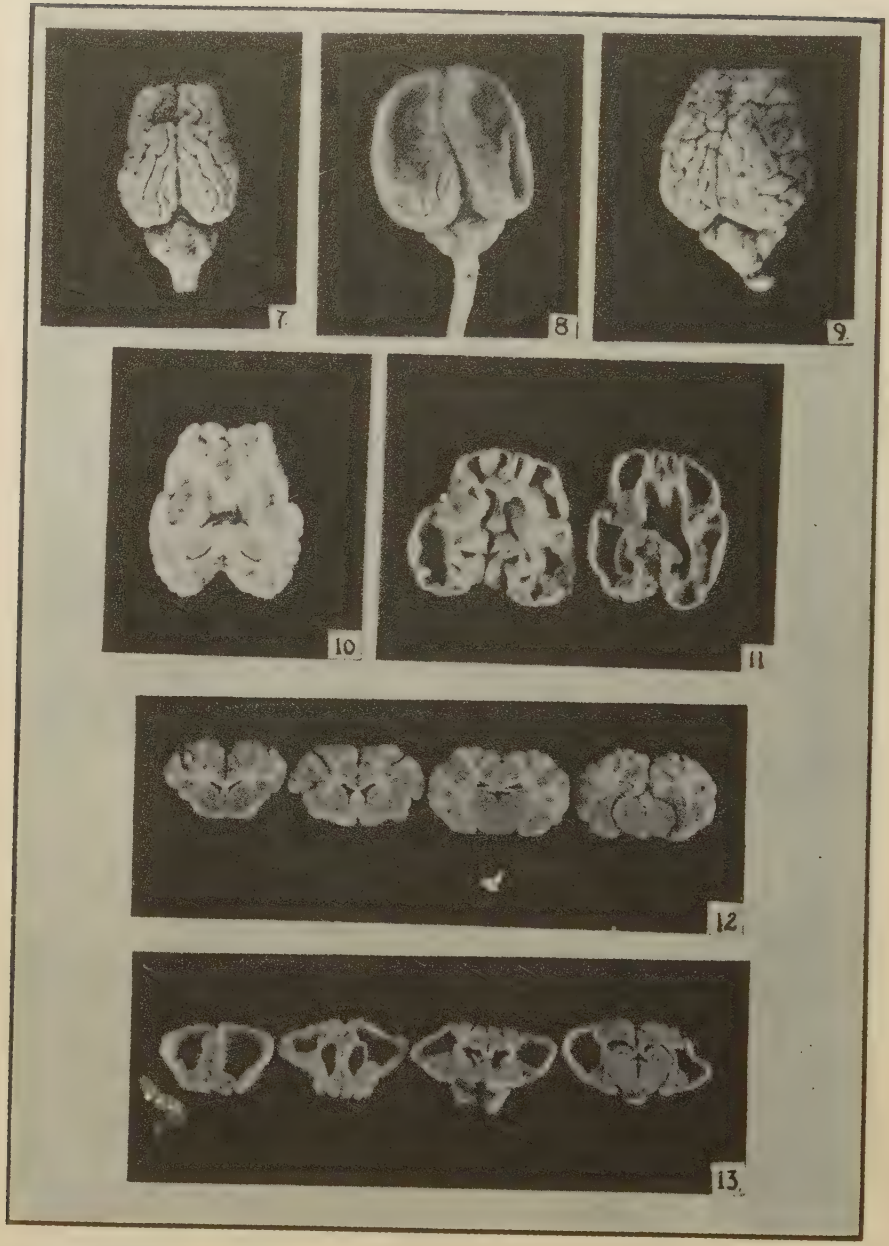
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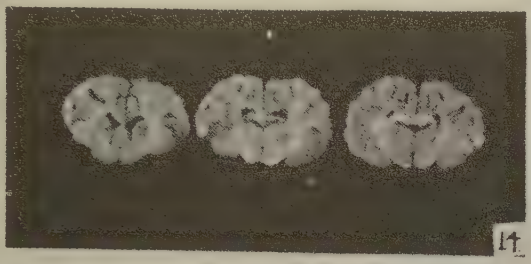
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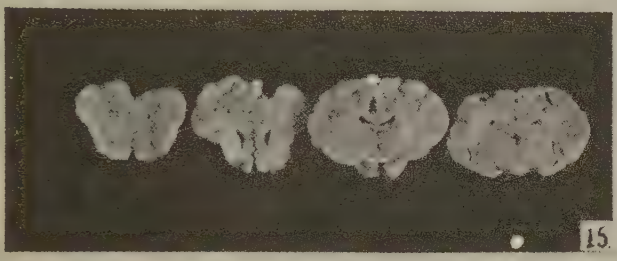
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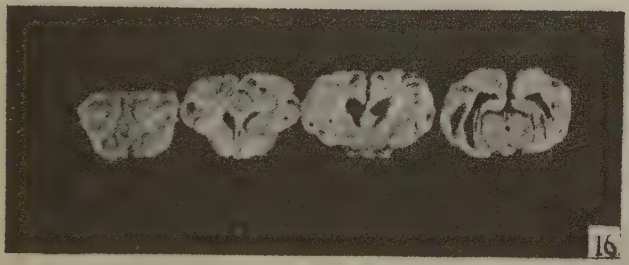
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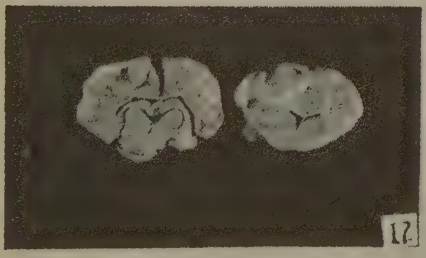
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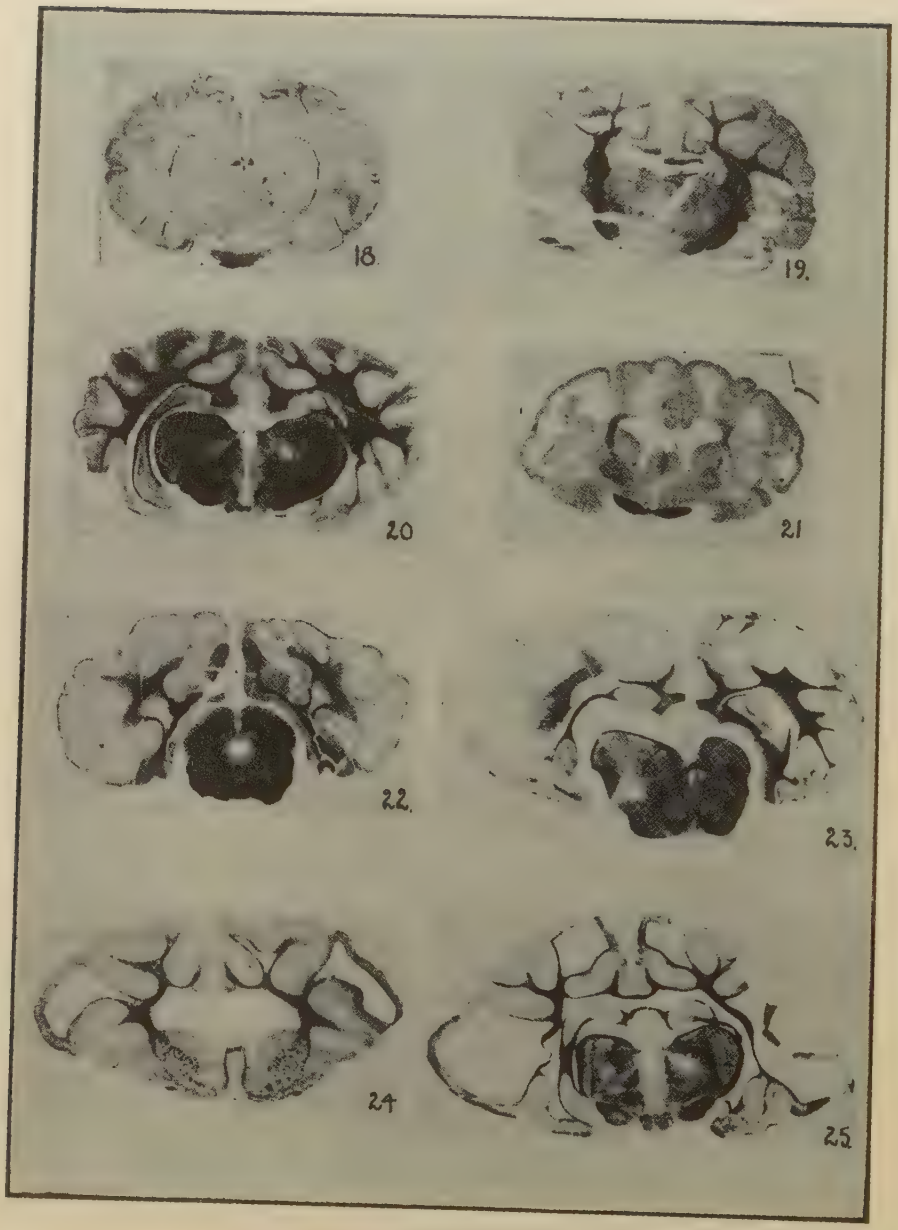
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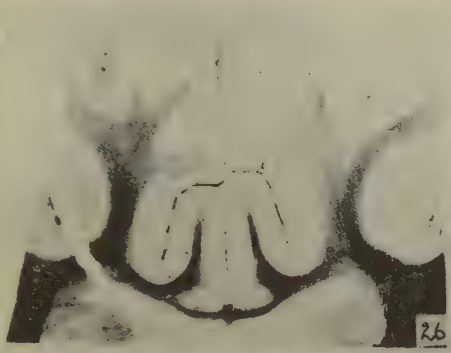
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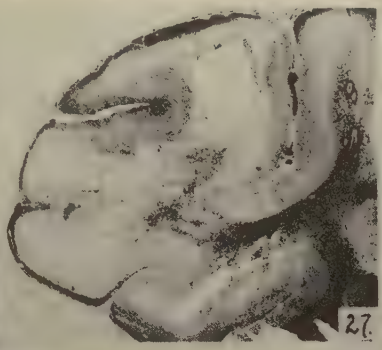
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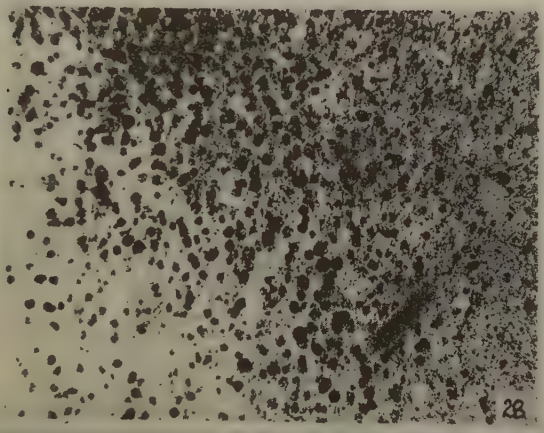
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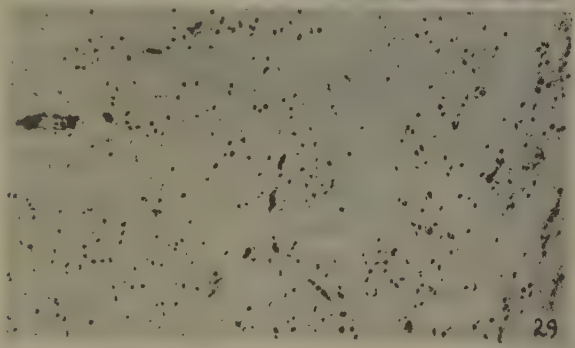
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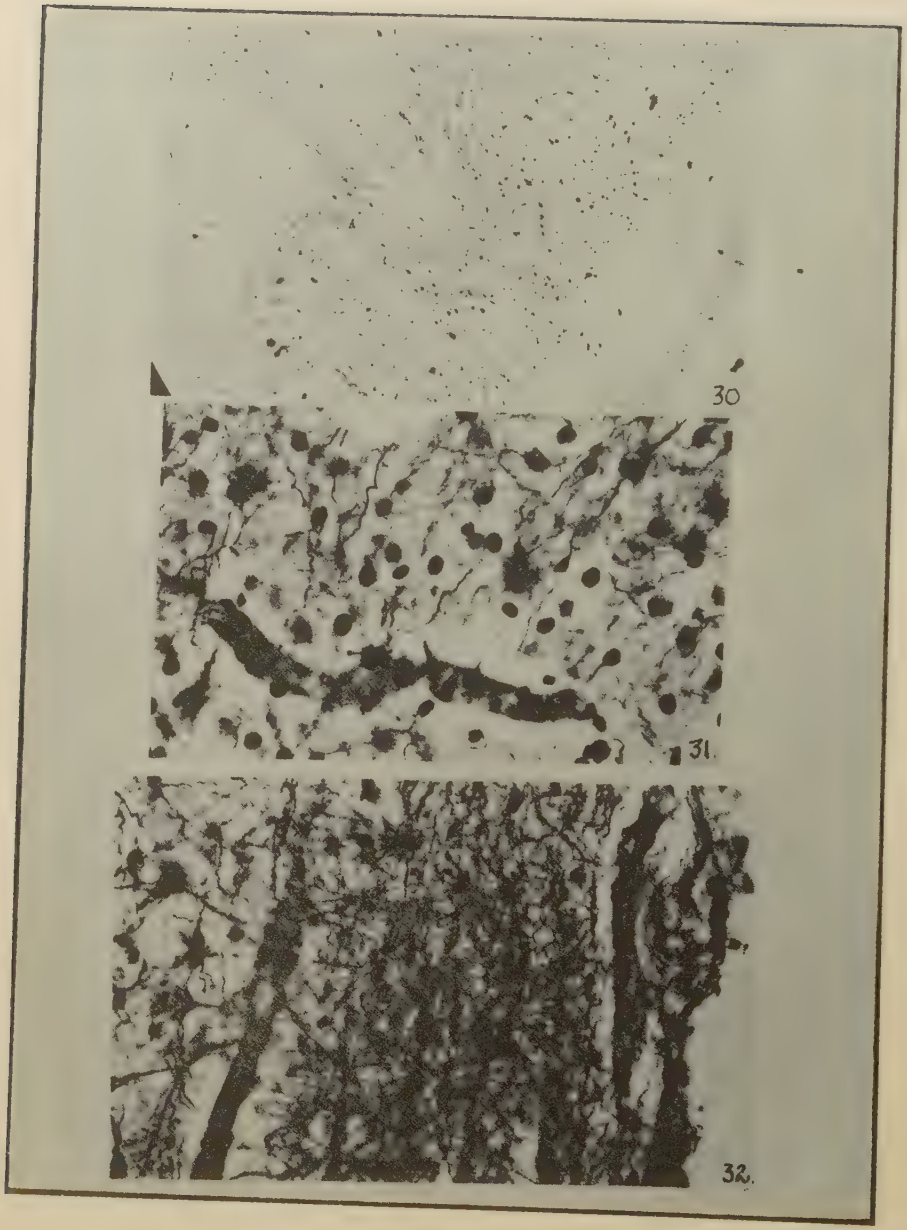
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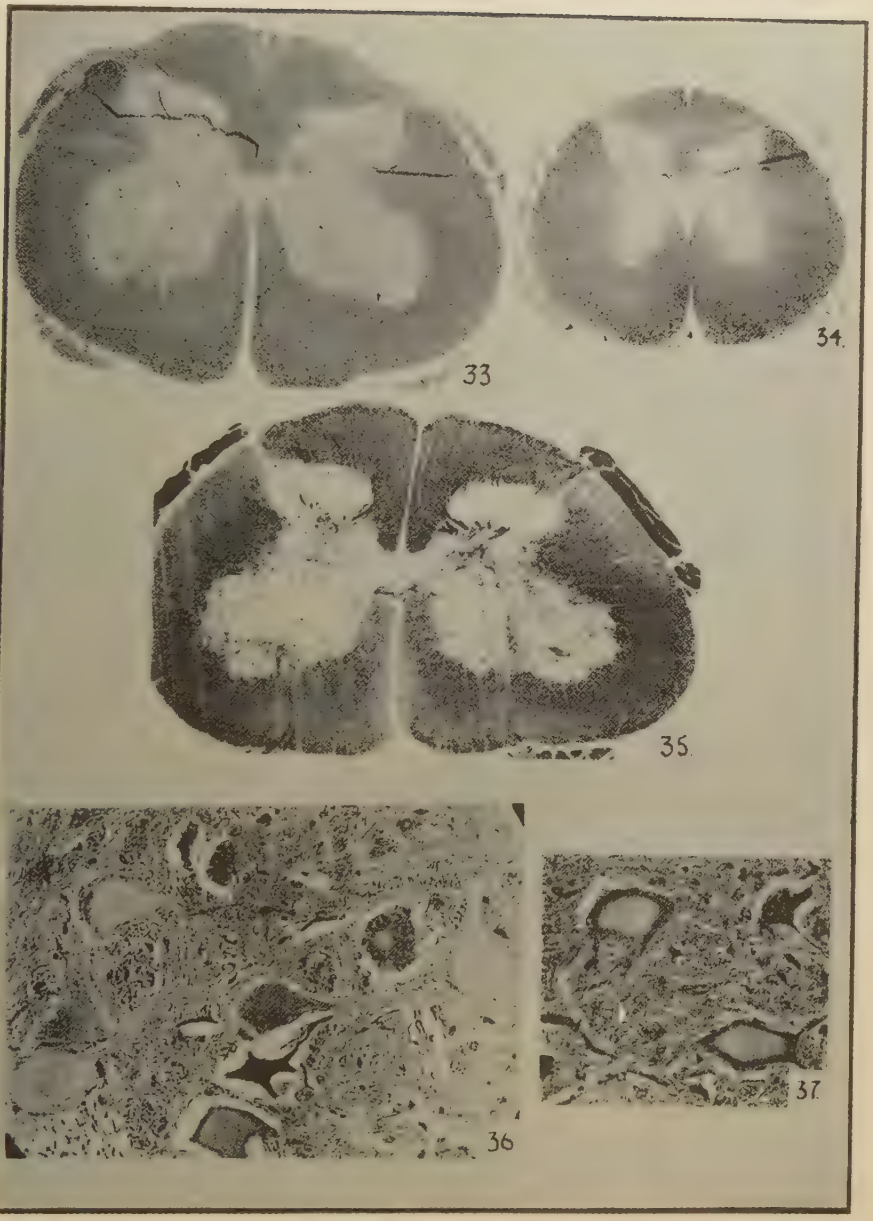
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CAPTIONS TO ILLUSTRATIONS

Figs, 1 to 6. A series of photographic strips enlarged from ciné-film to illustrate the symptoms of " Swayback," the strips to be observed from above down ; Fig. 1 shows four lambs with symptoms of varying severity : one lies helpless (see also Fig. 2) ; another is able to stand, while the animal at bottom left is in the act of toppling over ; Fig. 3 shows the straddled gait of a " Swayback " lamb ; Figs, 4, 5 and 6 show the futile efforts of severely affected lambs to stand.

Fig. 7. Brain, normal lamb, superior aspect ; for comparison with brains from " Swayback " cases in Figs. 8 and 9. The motor area in superior frontal gyrus is shaded on the left. About one-third natural size.

Fig. 8. " Swayback " ; dorsal aspect of brain showing the collapsed appearance of the cerebral hemispheres after removal from the skull ; the convolutions are blurred. P.M. Lamb No. 55/37. Three days old ; died. About half natural size.

Fig. 9. " Swayback " ; dorsal aspect of brain ; one of the only two cases which showed lesions unilateral in extent ; the convolutions on the right side appear wider and with rather indeterminate sulci ; this side felt soft and flabby, and on horizontal section the white matter showed diffuse gelatinous degeneration. P.M. Lamb No. 110/39. One of twins both affected at birth ; the other animal died when two days old and showed only small foci of degeneration in the cerebrum ; this animal was killed when 14 days old ; the symptoms observed at birth had progressed. About half natural size.

Fig. 10. Brain, normal lamb, horizontal section ; for comparison with " Swayback " brains (Fig. 11) ; frontal poles at top of photo. About half natural size.

Fig. 11. " Swayback " ; horizontal sections of cerebrum of two cases of " Swayback " showing gross destruction of white matter with extensive cavitation extending from frontal to occipital poles (bottom) ; the grey matter remains only as a thin shell around the cavities ; slight dilatation of the lateral ventricle. *Left photo* : P.M. Lamb No. 184/39 ; one day old ; died ; other twin of this animal was stillborn and showed no gross lesions in the brain. *Right photo* : P.M. Lamb No. 30/38. One of twins ; both affected at birth ; both killed when one day old ; other animal showed lesions of similar severity. About half natural size.

Fig. 12. Brain, normal ; two day old lamb ; series of coronal sections, frontal-occipital poles left-right ; for comparison with " Swayback " brains in Figs. 13 to 17. About one-third natural size.

Fig. 13. " Swayback " ; series of coronal sections as in Fig. 12 ; extensive symmetrical destruction of cerebral white matter with marked cavitation, wasting of corpus callosum and relative preservation of the grey matter. P.M. Lamb No. 15/38. Five days old, died ; this animal is seen on photograph, Fig. 4. About one-third natural size.

Fig. 14. " Swayback " ; coronal sections of brain as in Figs. 12 and 13 ; one of twins both affected at birth ; both able to walk but with straddled gait ; killed when five days old ; there are no lesions to be seen macroscopically ; foci of cerebral degeneration, cord degeneration and lesions in the red nucleus can be demonstrated histologically in such a case. About one-third natural size.

Fig. 15. " Swayback " ; coronal sections of the brain ; compare with other cases ; diffuse symmetrical gelatinous degeneration of the cerebral white matter extending from pole to pole ; demarcation between the grey and white matter is extremely blurred ; no cavitation ; this lesion is reminiscent of that seen in some cases of Schilder's encephalitis in children. P.M. Lamb No. 17/38. One of twins ; both severely affected at birth ; the other lamb died within a few hours of birth and showed gross lesions similar to those illustrated in Fig. 16 ; this case was killed when six days old ; the symptoms had progressed until the animal lay helpless. About one-third natural size.

Fig. 16. " Swayback " ; coronal sections of brain ; the appearances seen here might be regarded as a stage between those illustrated in Figs. 15 and 13 ; diffuse bilateral degeneration of cerebral white matter, rather gelatinous in type in the frontal poles with more evidence of liquefaction and cavitation in occipital region. P.M. Lamb No. 16/38. One of twins ; other animal died shortly after birth ; this animal was killed when two days old and is seen on photograph, Fig. 2. About one-third natural size.

Fig. 17. " Swayback " (Australian disease). Coronal sections of brain through occipital poles (left) and frontal poles (right) ; demarcation between the grey and white matter extremely blurred ; gelatinous type of degeneration without cavitation

comparable with many cases seen in this country and demonstrated in Fig. 15. (Specimen obtained through the courtesy of Mr. H. Bennetts, Western Australia). About one-third natural size.

FIGS. 18 TO 25. *Coronal section of the cerebrum from normal fœtal and young lambs and from cases of "Swayback." Weigert-Pal. Natural size.*

FIG. 18.—Normal fœtal lamb, 109 days (about one month before parturition). Compare the extent of myelination with that of a normal one day and three weeks old lamb; there is little evidence of myelination except in the capsules and optic chiasma. (Section presented by Mr. G. J. Romanes, School of Anatomy, Cambridge.)

FIG. 19. Normal one day old lamb. The central white matter is well myelinated and shows that within an interval of one month (compare Fig. 18) the process has been remarkably rapid.

FIG. 20. Normal three weeks old lamb. (Compare with Figs. 18 and 19.)

FIG. 21. "Swayback"; two day old lamb; diffuse bilateral demyelination of hemispheres to the extent of almost total loss of myelin with the exception of the optic chiasma. Sections from the occipital poles showed the same appearance. Lamb No. 6/35.

FIG. 22. "Swayback"; three weeks old lamb; no demyelination in the mid-brain; diffuse symmetrical demyelination of the hemispheres affecting particularly the subcortical zones; the fibres deep in the centrum ovale are densely stained. Lamb No. 8/35.

FIG. 23. "Swayback"; two weeks old lamb. Changes are similar to those seen in Fig. 22, but are definitely more unilateral in type. Lamb No. 16/35.

FIGS. 24 AND 25. "Swayback"; 18 days old lamb; liquefaction in this case has been extensive as evidenced by the gross cavitation; the process has primarily attacked the subcortical zones and spreads inwards; the deeper parts of centrum ovale, the capsules and the mid-brain show no demyelination. (Frontal poles, Fig. 24, occipital poles, Fig. 25.) Lamb No. 23/25.

FIG. 26. "Swayback"; coronal section from frontal region about level of superior frontal gyrus (motor area); corpus callosum and ventricle below. Diffuse demyelination affecting the subcortical zones to the summits of the convolutions. Lamb No. 9/35. Weigert-Pal. × 4.

FIG. 27. "Swayback"; occipital pole from same case as Fig. 26; beginning of porous cavity formation. Weigert-Pal. × 4.

FIG. 28. "Swayback"; same case as in Fig. 22. Sections of demyelinated area in subcortex. Marchi-Busch method. Granular accumulation of degenerated myelin fibres stained black; many of the larger fat globules are contained in phagocytic microglial cells. × 100.

FIG. 29. "Swayback"; section of demyelinated area showing extreme spongy nature of white matter; dilated perivascular spaces; absence of inflammatory cell infiltration; hæmotoxylin and eosin. × 100.

FIG. 30. "Swayback"; section of cerebral cortex outside a cavity, pia mater top left and lumen of cavity bottom right. Relative preservation of nerve cells; absence of any inflammatory cell infiltration; from same case as Figs. 25 and 26. Hæmotoxylin and eosin. × 120.

FIG. 31. "Swayback"; demyelinated area; occipital pole, same case as Figs. 24, 25 and 30 showing early fibrillary gliosis. Hortega's silver carbonate method; frozen section. × 400.

FIG. 32. "Swayback"; section of the subcortex covering a cavity from same case as Fig. 30; lumen on the right; intense gliosis. Hortega's silver carbonate method; frozen section. × 400.

FIG. 33. "Swayback"; spinal cord, lower cervical segment; degenerated myelin fibres stained black, present in ventral part of ventro-lateral columns near median fissure and dorsal part of same column adjacent to the dorsal horn of grey matter. From same case as Figs. 22, 28 and 29. Marchi-Busch method. × 8.

FIG. 34. "Swayback"; spinal cord, lower thoracic segment; same case as Fig. 33; degenerated fibres present in the same location as in the cervical region. Marchi-Busch method. × 8.

FIG. 35. "Swayback"; spinal cord stained by Weigert-Pal; compare with Fig. 33; note the paled area corresponding to the areas showing degeneration by the Marchi method. × 8.

FIGS. 36 AND 37. "Swayback"; lesions in the red nucleus from two separate cases; the large nerve cells show varying degrees of chromatolysis. Nissl-orange G method. × 400.

FURTHER OBSERVATIONS ON THE WILD RAT AS A CARRIER OF *BRUCELLA ABORTUS*.

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In a previous article (Bosworth, 1937) it was shown that wild rats may be infected with *Br. abortus* by the administration of grossly contaminated food and that they may then transmit the infection to other members of their species if kept in close confinement with them. In view of these findings, it was considered advisable to enquire as to the possible incidence of this type of infection among rats under natural conditions, particularly on premises where infected cattle are housed. At the time the article was written *Br. abortus* had been isolated from one of a small number of such animals examined, but the series has now been extended to include 167 rats caught on 19 farms in seven different counties. The farms were specially selected as those on which cases of abortion due to this organism had recently occurred among cattle. It may be recalled that whereas Karkadinovsky (1936) had reported a high incidence of infection under similar conditions among rats on farms in Russia, Menton (1937) had failed to find any evidence of its presence in a series which he examined in Staffordshire.

In the meantime other workers have published observations bearing on this problem. Sandholm (1938) conducted a series of experiments which were mainly designed to ascertain the length of time during which *Br. abortus* is to be found in the fæces of rats after they have eaten contaminated food. and from these experiments he drew certain conclusions as to the chances of infection being produced in the rats themselves as the result of such feeding. He succeeded in demonstrating the organism, for periods varying up to seven days, in the fæces of a proportion (5 out of 30) of animals which had eaten an infective meal and thus showed that rats are potential excreters of infection and may play a part in spreading it under natural conditions. On the other hand, he found little evidence to indicate that the organism, when ingested, is apt to produce infection in them, for he was unable to recover it in culture from the tissues of his experimental animals and found that only two of them (out of 29) reacted positively to the agglutination test though two others showed a low titre (1/10) which he regarded as of doubtful significance. It must be borne in mind, however, that they were all killed off at intervals of from one to seven days after exposure, with the exception of six individuals which were allowed to live for periods varying from 8 to 18 days. Under these circumstances the results of the cultural and serological tests on which he relied are not entirely convincing, for it is customary and, in the writer's experience, necessary, to allow a considerably longer period

to elapse with rats, as with guinea-pigs, before one can be sure that they have not become infected with *Br. abortus* after they had been exposed to it experimentally.

Fitch and Bishop (1938) reported one case of natural infection in a series of 69 wild rats which they examined.

Incidence of Natural Infection.

For the purposes of this investigation, arrangements were made to obtain a supply of rats from selected farms where the herds were severely affected and cases of abortion were of frequent occurrence. The rats were caught in the cowsheds and adjoining buildings where they were likely to have been in contact with infected cattle. Owing to the difficulty of arranging for them to be taken alive and transported direct to the laboratory, it was not possible to test them for the presence of *Br. abortus* agglutinins and as they were frequently quite unfit for cultural examination by the time they were received, it was decided to rely mainly on the results of guinea-pig inoculation for the detection of *Br. abortus* infection. The guinea-pigs used for this purpose were first treated with serum to protect them against infection with the common types of anærobic bacteria likely to be present in the test material. From each rat, pieces of spleen, liver, lung and kidney, together with lymph glands of the head and mesentery, were mixed, ground up with sterile sand and suspended in saline. After being lightly centrifuged to remove coarse particles, the resultant suspension was used to inoculate two guinea-pigs subcutaneously. In some cases material from two or three rats was pooled and injected into four or six guinea-pigs respectively. These were tested for agglutinins after a month's interval and again two or three weeks later when they were killed for the purpose of examining their organs by cultural methods for the presence of *Br. abortus*.

The results of this investigation were almost consistently negative for, of the series of 167 rats, only one was shown to be harbouring *Br. abortus* and this was the animal to which reference has already been made in an earlier article (Bosworth, 1937). The finding of such a low incidence of infection among rats living in surroundings in which they must have been exposed to it confirms the results which have been recorded by Menton and by Fitch and Bishop. From this it will be seen that other workers have found no evidence to support the view expressed by Karkadinovsky that rats are prone to become active carriers of *Br. abortus* and thus play an important part in its dissemination. There may have been special circumstances, which cannot yet be explained, to account for a high incidence of infection among them in his particular locality but until his experience has been shown to be other than a rare occurrence there is no need to take too serious a view of the position. Karkadinovsky cited an instance of what he alleged to be "incontestable evidence" that a series of cases of undulant fever in man

was due to the agency of affected rats, presumably as the result of food contamination. It will be seen later in this article that an attempt to transmit infection to cows by exposing their food for several months to contamination by affected rats proved unsuccessful.

Before passing to that aspect of the question, however, it is proposed to deal quite briefly with two other types of infection met with in examining this series of rats.

(a) *Leptospira icterohæmorrhagiæ.*

Under the circumstances which have been outlined the inoculation of thick suspensions prepared from the organs of the rats was well tolerated by the experimental guinea-pigs and remarkably few deaths from intercurrent disease occurred among them. Five of them, however, died from *Leptospira icterohæmorrhagiæ* infection and showed typical lesions of that condition. Since only one of a pair inoculated from a single rat succumbed in each case, it follows that five of the rats, but no more, were shown to be carrying the Leptospira, a very much smaller percentage than had been anticipated. It is known that there is a wide variation in the percentage of rats found to harbour Leptospira in different localities of this country (Smith, 1938) and quite possibly there are fewer carriers in rural areas than in town sewers, but another factor to be considered concerns the period of survival of the organism in the tissues after death of the host. Smith failed to demonstrate virulent Leptospira in rats which had been killed and then brought to the laboratory within 24 hours of death, and surmised that this was due to the fact that, when death occurs, the barrier established by kidney cell membranes between the immune bodies in the serum and the Leptospira in the tubules breaks down and the Leptospira are killed off. In the present investigation, however, most of the rats were examined later than 24 hours after death and two of the five which proved to be harbouring the Leptospira had been dead for more than 48 hours. Moreover, the writer can recall several instances in which it has been possible by guinea-pig inoculation to establish the diagnosis of leptospiral jaundice in dogs which had been dead for at least as long a period but in none of these cases is it known whether or not the rats or the dogs in question had developed immune bodies. Thus, while it is apparent from Smith's results that a certain indication of the presence of Leptospira is obtainable only as the result of examination made immediately after death, it may be possible in some cases to demonstrate the organism at a much later stage and this should be attempted whenever necessary.

(b) *Mycobacterium tuberculosis.*

On two occasions batches of guinea-pigs which had been inoculated with the tissues of rats obtained from separate farms presented lesions of generalised tuberculosis when killed for examination six weeks later. The distribution of these lesions corresponded to

that which follows the subcutaneous injection of tubercle bacilli. A culture of the organism isolated from one of the second batch of affected guinea-pigs was kindly examined by Dr. A. Stanley Griffith who found it to be a bovine strain of full virulence. The finding of lesions of tuberculosis in the experimental guinea-pigs was quite unexpected for although Koch and Rabinowitsch (1907) had reported the isolation of avian tubercle bacilli from rats and mice caught in infected poultry yards, there appear to be no recorded cases of rats acting as carriers of the bovine bacilli. Indeed the workers of the Royal Commission on Tuberculosis (1907) found that these animals exhibited a remarkable degree of tolerance towards tubercle bacilli, both human and bovine, and that it was necessary, in most cases, to inoculate very large doses of culture in order to set up infection in them. Once the bacilli had established themselves, however, they tended to multiply extensively in the various organs of the body and to persist there in large numbers, but without causing more than a slight degree of tissue damage. Hence the formation of even very small tubercles was a rare occurrence but it was not uncommon to find areas of collapse and consolidation of the lung tissue. Attempts to infect rats by feeding enormous doses of bacilli failed to set up lesions in more than a very low percentage of cases.

It is not surprising, in view of these findings, that gross lesions were not observed in the two naturally infected rats met with in this investigation or that these animals were not suspected of being tuberculous until the guinea-pigs which had been inoculated with their tissues came to be examined. The fact that they were infected is, however, of interest and may be of some importance, depending on the extent to which infection is present among the rat population as a whole. So far as can be judged from the results recorded here, this is probably quite low, but a much wider survey is necessary to afford an index of the real position.

An Attempt to Transmit *Brucella abortus* from Rats to Cattle.

An experiment was carried out on a small scale to test the possibility of setting up infection in two cows by exposing their food to contamination by rats which were known to be carriers of *Br. abortus*. It was originally intended to keep the latter in the same building as the experimental cows, as would happen under farm conditions, but this idea had to be abandoned on account of the difficulty of preventing their escape from the premises available. They were therefore housed separately in an isolation box with a concrete floor where they were provided with old iron pipes containing pieces of sacking and covered with a heap of straw, in which to lie up. In addition to being supplied with water and occasionally with a little green food, they had free access to a mixture of bran, oats and cake which was spread out on the floor so as to be exposed to constant contamination by their droppings before being used for feeding the cows. This was collected twice a week and replaced by a

fresh supply. For the purpose of this experiment seven adult and seven half-grown rats were inoculated with a young culture of a virulent strain of *Br. abortus* in quantities estimated at 500 million organisms for each adult and one-tenth that dose for each of the others. They were maintained as a colony for three months during which they had access to the whole of the concentrated food supplied to the cows. Two of the young rats were found dead during the first week, but at the end of the experiment the numbers had increased to 18. These were killed and examined with the results shown in the following table, from which it will be seen that *Br. abortus* was recovered from seven of the surviving original members of the colony and that two of those born during the experiment showed evidence of having become infected.

Rat. No.	*Description.*	*Titre, 30/5/38.*	*Spleen Culture.*
	Original.		
1	Infected 28/2/38	80	+
2	,,	160	+
3	,,	0	—
4	,,	20	+
5	,,	0	—
6	,,	0	—
7	,,	80	+
8	,,	0	—
9	,,	320	+
10	,,	160	+
11	,,	20	—
12	,,	40	+
	Born during experiment		
13	,,	0	—
14	,,	40	—
15	,,	0	—
16	,,	0	—
17	,,	0	—
18	,,	80	+

It was thus clear that the concentrated food consumed by the cows during the experimental period of three months had all been exposed to contamination by infected rats. The cows in question were selected so as to ensure, as far as possible, that they were not only free from Brucella infection but had never previously been exposed to it. One, an Ayrshire, was taken from the Institute's herd which consisted of animals purchased as calves and tested at intervals over a period of more than five years without a reaction ever having been observed. She was in milk but not in-calf. The other, a Red Poll, was bought from a self-contained herd which had a clean record when tested in 1935 and had shown a very satisfactory breeding history in the meantime. The herd was tested again early in 1938, immediately before the animal was purchased, and again nc reactors were found. This cow was then in the eighth month of pregnancy.

Before the experiment was commenced milk samples from both cows were tested on two occasions by cultural methods and by guinea-pig inoculations for the presence of *Br. abortus* and the whey was examined for agglutinins. The results were all negative.

From March 1st, 1938, the animals were given a full ration of concentrated food to which the infected rats had been allowed access as described above. The feeding was continued for three months during which period, and for some weeks afterwards, blood and milk samples were tested at intervals from April 1st onwards. In the case of the Red Poll cow, ten blood tests and five milk tests were made between then and the end of October. No agglutination occurred in a 1 : 20 dilution of serum or of whey and *Br. abortus* was not found in the milk. The cow gave birth to a healthy calf on April 18th. The fœtal membranes, which appeared to be normal, were examined for the presence of *Br. abortus* by making smears and inoculating guinea-pigs with the material from the cotyledons. The results were negative. The milk of the Ayrshire cow likewise remained above suspicion but a slight rise in her blood titre occurred at the end of a month and was at first regarded as a possible indication of infection. As it did not become more marked, however, but soon began to fall back again, it was thought to be of doubtful significance. The cow's serum which, up to the commencement of the experiment had consistently shown a trace of agglutination in a 1 : 20 dilution, gave a distinct, though not complete, reaction at 1 : 40 a month later. After the second month only a trace was visible in the latter dilution and at the final test at the end of the fourth month, just before the animal was killed, the reaction in the 1 : 20 dilution was only slightly more marked than it had been originally.

As the cow had shown some fluctuation in blood titre and had developed a hygroma of the left knee, which was causing lameness, it was decided to have her slaughtered and to examine her organs for the presence of *Br. abortus.*

Representative samples of material were collected from the following sites:—

1. Submaxillary lymphatic glands.
2. Pharyngeal glands.
3. Bronchial and mediastinal glands.
4. Mesenteric and colic glands (three samples).
5. Liver and portal glands.
6. Gall bladder.
7. Spleen.
8. Uterus.
9. Iliac glands.
10. Supramammary glands.
11. Prescapular, popliteal and precrural glands.
12. Hygroma fluid and loose bodies (so-called joint mice).

The pieces of tissue were dipped in alcohol and flamed before being ground up with sterile sand and suspended in saline. The suspensions were centrifuged lightly to remove coarse particles and each was then inoculated into six guinea-pigs, which were killed and examined after an interval of six weeks. The usual agglutination and cultural tests for the presence of *Br. abortus* gave negative results in every case. There was thus a complete failure under the conditions of the experiment to demonstrate the transmission of infection to cows by exposing their food to contamination by rats which were known to be carriers of it. When a slight rise in the blood titre of one animal was first observed it seemed possible that the attempt might turn out to be successful, on the assumption that the rise was in the nature of a specific response to the ingestion of *Br. abortus* with the food. If this was the real explanation, however, it would appear that any invasion of the tissues by the organism that may have occurred was soon overcome, as no evidence of it could be found at the time the *post-mortem* examination was made.

While it would be unjustifiable to conclude from the results of a small experiment of this kind that there is little, if any, danger of rats spreading infection among cattle, it may be inferred that the risk of this happening is more remote than it had at one time appeared to be. The food consumed by the experimental cows during a period of three months was continually exposed to contamination by an infected colony and the chances of transmission were therefore much more favourable than they would ordinarily be under natural conditions, in view of the low incidence of infection among rats reported by all investigators with the exception of Karkadinovsky. It seems, therefore, that, as a general rule, rats are not to be regarded at all seriously as reservoirs of infection, but since Sandholm has shown that the organism is liable to pass through their alimentary canal and be excreted in their fæces for several days after the intake of infected food, there is a risk that, by acting as mechanical carriers, they may play some part in its dissemination.

Conclusions.

1. The incidence of *Br. abortus* infection among rats in this country was found to be low. The organism was recovered once only as the result of examining 167 individuals caught on infected farms.

2. It was not possible to determine the percentage of carriers of *Leptospira icterohæmorrhagiæ* in this series of rats as they were not examined for some time after death. In three instances, however, the Leptospira was found to have remained alive in the tissues for more than 24 hours, and in two others for more than 48 hours, after the rats were killed.

3. Two of the rats were carriers of tubercle bacilli. One of the strains concerned was shown to be of the bovine type; the other, though not fully examined, was probably identical.

4. An attempt to convey *Br. abortus* infection to cows by exposing their food, for a period of three months, to contamination by infected rats was unsuccessful.

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ANTIGENIC VARIATION IN A STRAIN OF *CL. WELCHII* TYPE C. (*B. PALUDIS*)

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Evidence is accumulating from different sources to the effect that an alteration in the original antigenic structure of certain strains of *Cl. welchii* may result from their prolonged maintenance in artificial media. Thus, Dalling (1934) in collaboration with Montgomerie and Rowlands (1934) demonstrated that a Type B culture had, over a period of years, lost its power to produce D toxin (X factor of Wilsdon (1931), epsilon toxin of Glenny *et al.* (1933)) and had become antigenically indistinguishable from a Type C culture. This was further confirmed by Mason (1935). In a recent review of the production of toxins by the *Cl. welchii* group as a whole, Dalling and Ross (1938) have shown that the above Type B culture, although antigenically a Type C, retains the fermentative and hæmolytic properties of a Type B; and on one occasion they found that a Type C culture failed entirely to produce C toxin (beta factor of Glenny *et al.* (1933).

Mason (1935) showed that on one occasion a Type D culture was capable of immunising guinea-pigs against Type B toxin, but he was unable to repeat this result with the same culture later.

Borthwick (1937) reported that in her hands a recently isolated strain of Type D changed to Type A, subsequently reverted to Type D and then again to Type A.

The present article is a record of a culture of *Cl. welchii*, Type C, which appears to have altered considerably in antigenic structure since its primary isolation a number of years ago.

Methods.

Cultures were grown and maintained in cooked meat (horseflesh broth) containing 1 per cent. Bacto peptone and of *p*H 7·6 to 7·8. All cultures, except where otherwise stated, were incubated for from 18 to 20 hours at 37° C.

Culture fluid for use in toxicity and toxin-antitoxin tests was first centrifuged at 3,000 r.p.m. for 30 minutes. In those instances in which the dry toxin was used this was prepared by dissolving 100 mg. in 5·0 c.c. of sterile normal saline.

Toxin-antitoxin mixtures were kept at laboratory temperature for at least 30 minutes before intravenous inoculation into mice of 20 to 24 gm. weight.

The following antitoxins were used: Type A, "GG3269," Type C, "C1821," and "R3589," kindly supplied by the Wellcome Physiological Research Laboratories.

Type B, "Adra." Prepared in a hore at this Institute and capable of neutralising the toxin of *Cl. welchii,* Type B, in every phase of its growth.

Type C, "CT40 serum." Prepared in a horse at this Institute.

Type D, "H35/4." Prepared in a horse at this Institute from a strain of *Cl. welchii,* Type D, isolated from an acute case of "grass sickness."

Experimental.

A culture of *Cl. welchii,* Type C, which had been originally isolated by McEwen (1930) and labelled "*B. paludis* CT40" was received from Professor T. Dalling in 1933. In August of the same year the m.l.d. of culture fluid for mice was approximately 0·0001 c.c., and 0·00025 c.c. was neutralised only by *B. paludis* antitoxin obtained from the Wellcome Research Laboratories. At this period, therefore, the culture was a fully toxic strain of *Cl. welchii,* Type C.

No further records of this strain are available until June, 1935, when the m.l.d. of culture fluid had increased from 0·0001 c.c. to 0·0025 c.c., but 10 m.l.d. were still neutralised only by *B. paludis* antitoxin.

In 1936, dry toxin, and later, antitoxin was prepared in a horse at this Institute from CT40. The culture was subcultivated rapidly in meat broth prior to the preparation of toxin, the m.l.d. varying from 0·01 c.c. to 0·005 c.c. of culture fluid. The m.l.d. for mice of the dry toxin (precipitated from culture by the addition of ammonium sulphate) was 0·5 mg. and 4·0 mg. was neutralised by 0·004 c.c. of Type C antitoxin C1821. Antitoxin prepared in a horse (CT40 serum) neutralised the same dose of toxin in a quantity of 0·002 c.c.

In 1937, Professor Dalling examined a sample of CT40 serum and drew our attention to the fact that it had a very low C antitoxin value. The serum was re-tested in mice, using 4·0 mg. (< 10 m.l.d.) of dry CT40 toxin as a "test dose." It was found that this amount was neutralised by 0·002 c.c. of CT40 serum, by 0·002 c.c. of Type C antitoxin "R3589," and by 0·04 c.c. of Type B antitoxin "Adra." The tests were repeated, with the same sera but a different toxin (C11138 prepared from *Cl. welchii,* Type C, W49, received from Dr. R. F. Montgomerie in 1937) whereupon it was found that 10 m.l.d. were neutralised by 0·002 c.c. of R3589, by 0·0004 c.c. of Type B antitoxin "Adra" and not at all by 0·1 c.c. of CT40 serum. 0·1 c.c. of the latter neutralised 1 m.l.d. but not 5 m.l.d. of the new toxin.

The strain used in the preparation of the dry CT40 toxin was labelled "T" and subcultivated twice daily for ten days and toxin/antitoxin tests performed in mice. The m.l.d. was approximately 0·025 c.c. of culture fluid, and it was found that 0·25 c.c. was neutralised by Type A antitoxin GG3269 as well as by Type C antitoxin R3589. The strain was subcultivated daily over a period of

three weeks and re-tested as in Table I. The m.l.d. was approximately 0·05 c.c.

Table I.

Dose of Antitoxin.	*Dose of Culture Fluid.*	*Result.*
0·1 c.c. "A" GG3269	0·5 c.c.	+L L
	0·1 c.c.	L L L
0·1 c.c. "C" R3589	0·5 c.c.	L L L
	0·1 c.c.	L L L
0·1 c.c. "D" H35/4	0·5 c.c.	++L
	0·1 c.c.	L L L

Three normal guinea-pigs were each given subcutaneously 5·0 c.c. of Type A, C and D antitoxins respectively, and on the following day, along with an uninoculated control, they received 0·5 c.c. of an overnight growth of CT40 "T" intramuscularly. The immunised animals had suffered no ill effects after a period of four days, while the control died overnight from gas gangrene.

CT40 "T" appears, therefore, to have lost much, although not quite all, of its power to produce C toxin (Z factor of Wilsdon, beta toxin of Glenny) in that Types A and D antitoxin will protect a certain proportion of mice against approximately 10 lethal doses of culture fluid and will protect guinea-pigs against infection after the intramuscular inoculation of whole culture.

As a result of these findings it was considered desirable to obtain the CT40 strain from a number of different sources with a view to determining if other strains obtainable had also deteriorated. Accordingly, an attempt was made to isolate the CT40 strain from dry unfiltered material precipitated from culture fluid by ammonium sulphate in 1933. A culture of *Cl. welchii* was isolated and this was labelled CT40 "V." A culture sealed in alkaline egg medium in 1935 was similarly cultured and labelled CT40 "W" and four strains were supplied by other laboratories and labelled respectively CT40 "U," "X," "Y" and "Z."

The seven sub-strains were subcultivated daily for ten days and toxin-antitoxin tests performed in June, 1938. This procedure has been repeated twice, in December, 1938, and in June, 1939. In the interval between tests the cultures have been stored in the dark at laboratory temperature (15° to 17° C.) in unsealed tubes of meat broth. The approximate m.l.d. for mice of each strain has varied on each occasion between 0·05 c.c. and 0·0075 c.c. of culture fluid, with the exception of CT40 "Z" which has behaved throughout as a true Type C with an m.l.d. in the region of 0·001 c.c.

TABLE II.

Antitoxin.	*Dose of Culture. c.c.*	T. *May,* 1938.	T. *Dec.,* 1938.	T. *June,* 1939.	U. *May,* 1938.	U. *Dec.,* 1938.	U. *June,* 1939.	V. *May,* 1938.	V. *Dec.,* 1938.	V. *June,* 1939.
0·1 c.c. "A" GG3269	0·5		++	++	+++	++	++	++	++	++
	0·1		++	LL	+++	++	LL	LL	LL	LL
0·1 c.c. "C" R3589	0·5		L	L	LL	LL	LL	LL	LL	LL
	0·1		L	L	L	L	L	L	L	L

Antitoxin.	*Dose of Culture. c.c.*	W. *May,* 1938.	W. *Dec.,* 1938.	W. *June,* 1939.	X. *May,* 1938.	X. *Dec.,* 1938.	X. *June,* 1939.	Y. *May,* 1938.	Y. *Dec.,* 1938.	Y. *June,* 1939.
0·1 c.c. "A" GG3269	0·5	+L	++	LL	++	++	++	++	+L	++
	0·1	LL	LL	LL	LL	LL	LL	LL	LL	LL
0·1 c.c. "C" R3589	0·5	LL	LL	LL	LL	LL	LL	LL	LL	LL
	0·1	L	L	L	L	L	L	L	L	L

The guinea-pig test was repeated with the six new cultures, the result being that Types A and D antitoxins protected against infection by every strain except CT40 "Z" which was neutralised only by Type C antitoxin.

Each strain was plated on blood agar and incubated anærobically overnight in a Mackintosh and Fildes' jar. Five colonies from each strain were picked into meat broth tubes which were incubated overnight. 0·5 c.c. of fluid from each of the resultant cultures was tested against 0·1 c.c. of Types A and C antitoxins, along with suitable controls. According to this arbitrary classification, "T," "V" and "W" gave one Type C and four Type A colonies, "Y" two Type C and three Type A, "X" five Type A, and "U" and "Z" each five Type C. The typing of the Type A cultures was confirmed, and the Type C cultures were tested for toxicity. None was lethal to mice in a dose smaller than 0·05 c.c. of culture fluid except strain "Z" which killed in a dose of 0·0005 c.c.

An attempt was made to increase the toxicity of CT40 "T" by animal passage. A guinea-pig was given 1·0 c.c. of actively growing culture intramuscularly. The animal died overnight and 1·0 c.c. of exudate from the gangrenous lesion was inoculated into another guinea-pig. This was repeated five times. Exudate from the last guinea-pig was plated on blood agar and incubated anærobically. Ten colonies of *Cl. welchii* were picked into meat broth, subcultivated rapidly for a few days and tested in mice. None was lethal in a dose of less than 0·05 c.c. of culture fluid.

Discussion.

It is well recognised that prolonged maintenance in artificial media tends to lower the virulence and toxigenicity of bacteria and that this is frequently associated with changes in antigenic constitution.

The CT40 strain of *Cl. welchii* described in this paper was shown to contain in a marked degree the Z factor of Wilsdon (beta toxin of Glenny) when originally examined, whereas after maintenance on artificial media for six years, much if not all of this factor had disappeared. This is but a continuation of an hypothesis advanced by Mason (1935) that *Cl. welchii*, Type C, may at one time have been capable of producing D toxin (X factor of Wilsdon, epsilon toxin of Glenny) but has, like the Type B culture described by Dalling (1934) lost this power. The CT40 strains would appear to have continued this process until a number of them are now, antigenically at least, virtually Type A.

Summary

The gradual loss of ability, over a period of years, by a culture of *Cl. welchii*, Type C, and by cultures of the same strain obtained from different sources, to produce C toxin (Z factor of Wilsdon, beta toxin of Glenny) has been demonstrated and briefly discussed. This confirms the observation of Dalling and Ross (1938) that a culture of *Cl. welchii*, Type C, may on occasion fail to produce C toxin.

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ALLERGY IN THE DOMESTIC ANIMALS

Survey of

(A) Factors Incidental to Domestication Probably Favouring Absorption of Native Food Protein.

(B) A Number of Disease Conditions Possibly Associated with Sensitisation to Food and Other Proteins.

By A. Brownlee, B.Sc., M.R.C.V.S.

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I.—Introduction.

When a foreign protein, such as egg white, heterologous serum, plant proteins, is introduced parenterally into the animal body either by inoculation or by absorption through the mucous membranes, no recognised symptoms are shown but the animal body may nevertheless react to the presence of the protein in such a manner that the

tissues become hypersensitised in a period typically of about ten days and, if, thereafter, a second dose of the same protein reaches the sensitised tissues, a reaction occurs and symptoms of mild or severe illness become manifest and even death may ensue. The nature and mechanism of diseases associated with hypersensitiveness to foreign protein is still obscure and certain differences exist between the naturally occurring diseases in human beings and artificially produced conditions with the result that several terms such as Allergy, Anaphylaxis and Atopy have been employed in an attempt to differentiate such conditions (*vide* Coca, Walzer and Thommen, 1931; Bray, 1937; Rowe, 1937). On a pathological basis the symptoms of allergy are caused by (*a*) smooth muscle spasm, (*b*) increased capillary permeability.

Variations occur in the readiness with which the tissues of the different animal species become sensitised. The guinea-pig is usually sensitised by one suitable dose, whereas mice, in many cases, require several doses before sensitisation is established (Burdon, 1937). The type of reaction produced also varies both with the species of animal and with the route of inoculation. Sensitised guinea-pigs, inoculated intravenously with a shocking dose of maximum efficiency die in 2 to 5 minutes with characteristic symptoms, largely respiratory. If the dose be given subcutaneously the course is much more prolonged and symptoms are quite different (Auer and Lewis, 1910). In the dog, on the other hand, there is a great fall in blood pressure and severe congestion of the liver but no respiratory involvement such as is found in the guinea-pig (Weil, 1917). Arthus (1903) found that if a rabbit be inoculated subcutaneously in the thoracic or abdominal regions with 5 c.c. of horse serum every six days, resorption occurs in a few hours after the first three inoculations but subsequent doses with the increasing degree of tissue sensitisation, were followed by increased œdema at the site of inoculation and after the seventh inoculation necrosis and gangrene occurred. The same result was obtained if the sensitising doses were given intraperitoneally and only the shocking dose given subcutaneously.

It is not essential that foreign protein as such be inoculated: certain carbohydrate radicals and certain drugs such as cincophen (Quick, 1934) may, when inoculated or absorbed, cause sensitisation. It is generally thought that such substances combine with proteins in the body, thus producing proteins which are foreign. Rosenau and Anderson (1907) found that the amino acids employed by them did not produce sensitisation in guinea-pigs and it is generally recognised that it is the intact native proteins which sensitise.

Once sensitised an animal will react to further stimuli with homologous protein, if this be given at spaced intervals. Immediately following a reaction there is a period of hyposensitisation during which no reaction to such stimuli will occur, but this phase passes and sensitisation becomes re-established. This is probably

one of the reasons why allergic conditions tend to be intermittent, the attacks being alternated with periods of freedom from symptoms. Later, if the causal factors be not removed, the attacks tend to become more chronic and continuous.

Ratner and Gruehl (1934) found that mature as well as young normal experimental animals could be sensitised and shocked by oral administration of protein foods. In the normal human subject unaltered food protein can be absorbed (Walzer, 1926 and 1927), but the large majority of individuals appear to be able to deal with this naturally absorbed protein satisfactorily without becoming sensitised or they may show cutaneous sensitivity for only a short time, after which sensitivity is lost (Du Bois, *et al.*, 1925). On the other hand, certain individuals become and remain sensitised to naturally absorbed protein and on re-exposure attacks of allergic disease may ensue. Such diseases in the human subject are asthma, hay fever, urticaria, eczema, migraine and some gastro-intestinal disturbances. There are also a number of conditions which are considered by some as possibly allergic in origin, *e.g.*, epilepsy (Spangler, 1932), arthritis (Pottenger, 1938) and gout (Burt and Gordon, 1939). A feature of many allergic conditions is that recovery from an attack often occurs without an ensuing period of convalescence. Allergic disease in man may be produced by absorption of foreign protein *via* the nasal mucosa in the case of pollen, animal epilations and vegetable dusts or from the alimentary tract in the case of food protein sensitisation. The tendency to develop allergy is generally recognised to be in the majority of cases hereditary. Bray (loc. cit., p. 89) states that "Statistical surveys have shown that the majority of allergics have one or more antecedents with some recognised form of allergy and that a certain proportion of the children will develop symptoms. Others of these children, while not manifesting any sensitivity themselves, may again transmit the tendency to their children." It is often exceedingly difficult to set up any of these conditions by inoculation or by natural exposure to a foreign protein even when this protein is known to be capable of acting as an allergen. Besides an hereditary diathesis some other, as yet undetermined, factors appear to be necessary for the production of clinical manifestations of these diseases. Bray (loc. cit.) reviews these possible concomitant factors and favours the view that acid-base disequilibrium plays a part.

Bray (loc. cit., p. 78) points out that up to the present it has not been shown definitely that a true germinal hereditary influence plays any part in anaphylactic conditions in animals. He records one possible instance in which cases of "hay fever" occurred in a herd of pedigree Jersey cattle. The disease did not affect cross-bred cattle kept under the same conditions. Wells and Osborne (1911) found that certain strains of guinea-pigs are not so susceptible to sensitisation as others. Simon (1938) considers that the animal species, other than man, have probably been inadequately studied with reference to possible allergic disease.

The objects of the present work are to draw attention to factors, incidental to domestication, which probably favour the absorption of native food protein and to consider some disease conditions, which may be associated with tissue reactions due to protein sensitisation and subsequent shocking.

(A).

II.—General Physiological Principles Involved.

The large majority of the diseases dealt with in this communication are attributed to disturbances in the mechanism of the digestion of food. It has long been recognised that one of the functions of the stomach is to commence the breaking down of proteins, but less emphasis appears to have been given to the potential danger to the organism of naked proteins not broken down before gaining access to the absorptive surface of the intestine. The organism tries to guard itself against this accident in the following ways:—

(*a*) The shape of the simple mammalian stomach with entrance and exit placed close together and the valve-like pyloric exit is such that solid, undigested food can be retained until thoroughly broken down.
(*b*) The gastric juice is strongly proteolytic.
(*c*) Milk is clotted so that the casein can be the more easily retained.
(*d*) The mechanism of thirst in the various animals may be related to this protective agency.

In the case of herbivorous animals with a small simple stomach, such as the horse, unchanged protein will doubtless pass into the intestine during the course of a grazing period, but presumably the stomach is capable of dealing with naked protein from ruptured plant cells and it is only protein, still enclosed within intact cellulose cell envelopes and thus unabsorbable, which passes on into the intestine in its native condition under optimal physiological conditions.

III.—Drinking Habits of Animals.

The various species of domestic animals appear to differ in their drinking habits. Tangl (1902) observed that horses given water *ad lib.* may drink before a meal but they have a greater tendency to drink at the close of a meal. The present author's observations on horses allowed water *ad lib.* confirm these findings. Linton (1927) found that "when the stomach was full, as after the completion of an ordinary feed, a large drink of water did not mix with the stomach contents at all but passed directly out along the lesser curvature of the stomach leaving the stomach contents intact. On the other hand, if only a small feed was given and then the horse allowed to drink a large quantity of water it did mix with the food and carried a small amount into the small intestine."

Brownlee (1939 (i)) found that cattle tended to drink water at the close of a meal. In a healthy heifer strictly rationed and fed

twice per day water was consumed at or near the close of a meal and very rarely at any other time (unpublished observation).

In the dog, on the other hand, Gregerson (1932) found that animals kept in cages, fed once per day and allowed water *ad lib.* drank water 2 to 5 hours after feeding and scarcely at all at other times. In a few observations made on leopards* fed once per day on flesh, the present author observed that these animals did not drink water until after over two hours from time of feeding.

The present author made observations on two healthy pigs which were being fed regularly three times per day and allowed water *ad lib.* These animals drank water in small amounts from time to time for several hours after a meal of solid food. Repeated observations on poultry showed that they tended to drink water immediately after the close of a meal (unpublished observations).

These times of drinking water probably have a relationship to the desire on the part of the organism to avoid naked protein being washed on to the absorptive surfaces which constitutes an added danger owing to the fact that water is not long retained in the stomach but passes on into the intestine. The horse overcomes the difficulty by drinking mostly at the close of a meal when the stomach is full and the food well compressed so that water does not tend to mix with it. In the ox, since food is very little masticated on first deglutition, most of the protein will still be enclosed in the cellulose-walled plant cells at the close of the meal. In the case of the dog, flesh and blood proteins would be free to mix with any ingested water, so this animal and possibly other carnivors wait for several hours, until such proteins are broken down before water is consumed. The omnivorous pig appears to steer a middle course and to drink only in small quantities so that at no time is there a rapid passage of water from the stomach. The pig's teeth are designed to crush food rather than to grind it so that the food enters the stomach in relatively large fragments and can thus be retained until broken down. Poultry under natural conditions like the ruminants swallow their food, both animal and vegetable, practically uncrushed so that at the close of a meal there is a minimum of free protein and also the ingested food is in the form of relatively large pieces which can be retained by the crop and gizzard (*vide* Brownlee, 1939 (ii)).

IV and V.—Influence of Unnatural Foods and the Influence of Milling of Foods.

The practice of giving horses, cattle and sheep, quantities of threshed cereal and other seeds and their by-products, when their natural food is bulkier material, and the practice of giving pigs and poultry an all-mash ration, when naturally their food is swallowed in larger units, does not permit of maximum efficiency of the stomach, as water consumed by animals so fed is practically certain

*I am indebted to the Zoological Society of Scotland for facilities in carrying out these observations in the Scottish National Zoological Park, Edinburgh.

to wash out from the stomach particles of food which are not yet dealt with physiologically. That animals, as a rule, thrive and produce when fed in this manner none will dispute, but individual animals often show disease manifestations under such circumstances.

VI.—Influence of Overfeeding and Irregular Feeding.

These are undoubted factors in the production of disease. Conditions of animal and crop husbandry render domestic animals liable to this contingency, often due to accidental circumstances. In the human subject Ratner (1928) obtained evidence that excessive consumption of particular foods by pregnant women led to sensitisation of the fœtus, thus pointing to an absorption of native protein from the alimentary tract under such circumstances.

VII.—Influence of the Kind of Food.

Sulzberger and Mayer (1931) found that guinea-pigs fed on dry winter fodder were more easily sensitised to arsphenamine and to paraphenylendiamine than were guinea-pigs fed on green summer food. It is well recognised in veterinary medicine that animals on winter feeding are more liable to certain diseases than animals at pasture. So also are the offspring of animals so fed.

The feeding of dog biscuits, with their high carbohydrate content, to dogs may be unphysiological: Voit (quoted by Von Noorden, 1907) found that, in dogs fed on a diet consisting solely of bread, there was retention of water in the tissues and that on changing to a meat diet, there was a great loss of water from the tissues.

(B).

VIII.—Acute Diseases Associated with Symptoms of Nervous System Involvement.

(*a*) *Milk Fever.*—This is a condition occurring in newly calved cows especially in high milk-yielding animals which are in good condition or which have been generously fed for a period prior to parturition; an easy delivery appears to render an animal more liable to attack. It is characterised by tetany, convulsions, coma, and death in a large percentage of untreated cases. Schmidt found that by insufflation of the mammary gland with air, rapid recovery, usually occurred. Dryerre and Greig (1925) postulated a parathyroid deficiency leading to faulty metabolism and diminished available calcium. Little and Wright (1925) working independently showed that a marked hypocalcæmia was present in this condition.

It has been shown that calcium therapy also generally brings about rapid recovery. The more basic ætiological factors have not been determined. Marek (1926) postulated that milk fever was due to anaphylactic autointoxication produced by proteins of the placenta or of the glandular cells of the udder. Small scale attempts to set up the condition experimentally by inoculation of cattle with placenta, colostrum and milk were unsuccessful. However, as

pointed out above, it is difficult to set up experimentally the naturally occurring allergic conditions. Also the likelihood of obtaining a positive result by such experimental methods will remain problematical until more is known about sensitisation in cattle. The extreme rarity of the condition in primipara may be due to sensitisation only occurring at time of parturition in which case all placental protein would generally be removed from the uterus by the time sensitisation had been properly established and the shocking doses would not be forthcoming until subsequent parturitions occurred. The tendency for recurrences of the disease in cows which have once been attacked is also suggestive of sensitisation. Rosenau and Anderson (1908) were able to sensitise and shock guinea-pigs by the inoculation of guinea-pig placental extract, and a similar phenomenon may be the basic ætiological factor in milk fever.

(*b*) *Lactation Tetany*.—This condition is most commonly met with in high milk-yielding cows within a few weeks after they have been allowed access to young succulent pasture of high protein content having been previously accustomed to bare pasture or to stall feeding. The symptoms are those of tetany and convulsions, with death in many cases (Blakemore and Stewart, 1932-33). In this condition a marked hypomagnesæmia is present and magnesium therapy appears to be of some value (Dryerre, 1932, Blakemore and Stewart (loc. cit.), Green, 1939, and subsequent discussion). In this condition also insufflation of the udder with air may be of value and if recovery is established it is often, as occurs also in most cases of recovery from milk fever, without convalescence (Lothian, 1931, Eaton, 1932, Godber, 1932). Green (loc. cit.) postulates that in this condition and in milk fever there is endocrine dysfunction and that a genetic factor may be involved in both.

The conditions of occurrence of this disease are such as would favour absorption of undigested protein. High-yielding cows drink most water and as excess water tends quickly to leave the rumen (Shalk and Amadon, 1928) this may be a factor, when combined with easily disrupted succulent food, in washing unchanged protein on to the absorptive surface of the intestine. The high protein content of rapidly growing pasture may also be a factor.

(*c*) *Hysteria*.—This has been defined as a condition of dogs characterised by paroxysmal attacks of deranged consciousness or sensibility usually manifested by crying, barking or running. Hewetson (1936) mentions allergy as a possible cause of this disease. The intervals of freedom from symptoms between the attacks, the tendency to recur and the feature of recovery usually without a period of convalescence are all suggestive of an allergic condition. Certain breeds of dogs appear to be more liable than others and highly strung dogs and in-bred dogs seem to be more susceptible. (Wilson, 1929; Hewetson, loc. cit.; Peele, 1937; Allan, 1937; Christopher, 1937). Feeding on starchy foods and the recent extensive use of dog biscuits are considered by many to play some rôle

in the production of this disease. Hewetson advocates giving raw meat not too finely minced else it may pass through the stomach before being properly digested.

As pointed out above, the feeding of dogs solely on bread led on a tissue hydration. McQuarrie *et al.* (1932), obtained evidence that water retention may be a factor in the production of human epilepsy and Spangler (loc. cit.) considers that epilepsy is probably an allergic disease.

IX.—Chronic Diseases associated with Symptoms of Nervous System Involvement.

(*a*) and (*b*) *Paralysis in Fowls and Scrapie in Sheep.*—Brownlee (1936), surveying literature on these two conditions, drew attention to many points of similarity between these two diseases. Both were characterised by (i) a fairly well defined age of maximum incidence, (ii) little or no systemic disturbance, (iii) an unknown ætiology, (iv) an association with generous feeding, (v) being prevalent in improved stock and associated with an hereditary diathesis. Since this publication another point of similarity has been brought to the author's attention. Cuilléand Chelle (1936 and 1938) in small scale experiments have found that scrapie can be set up experimentally in sheep after a long incubation period (11 to 22 months) by the inoculation of suspensions of brain and cord of naturally affected sheep. Pappenheimer *et al.* (1929), succeeded in setting up fowl paralysis in a percentage of experimental fowls by inoculating suspensions of central nervous system material. Here also there was a long incubation period (2 to 4 months). Other workers on fowl paralysis have obtained similar results. The fact that the artificially produced diseases manifested themselves in only a percentage of those inoculated even though precautions were taken to ensure that none of the inoculated animals were immune and that symptoms were not in evidence until several months after inoculation, even when the inoculation was made into the central nervous system, set these two conditions apart from known virus diseases and it is much more probable that the inoculum in each case contained an allergen and the experimental animals became sensitised.

It is a feature of scrapie that it is most prevalent in lowland stocks which receive artificial feeding. Fowl paralysis has become very prevalent recently and the recent increase in the system of rearing chickens almost entirely on mashes may account for the increased incidence. Intestinal parasites may also play a contributing rôle by increasing intestinal permeability. Warrack and Dalling (1932) found that fowl paralysis could occur in the absence of parasites.

(*c*) *Shivering and Stringhalt.*—Mitchell (1935) considers that these two conditions together with osteo-arthritic conditions commonly met with in horses, are all manifestations of one disease entity to which he gives the provisional name of "Rheumatic

disease of the horse." The ætiology is unknown. These disease conditions have long been considered to be hereditary (Williams, 1897; McCall, 1910: Robb, 1936) though Mitchell doubts this. Horses living under natural conditions do not suffer from these conditions and it is possible that the artificial feeding plays a rôle in their production and that the ætiological factor is protein sensitisation.

X.—Diseases associated with Infection with Anaerobic Bacteria.

Although in the diseases of this group such as braxy, associated with *Clostridium septique* infection, black disease associated with *Cl. œdematiens,* black quarter associated with *Cl. chauvœi*; lamb dysentery, enterotoxæmia and pulpy kidney associated with infection with different types of *Cl. welchii,* the generally fatal outcome is due to toxæmia resulting from the multiplication of these bacteria in the tissues or in the alimentary canal and that by immunising the animals liable to such attacks against the respective organism the mortality rate is cut down very considerably, it has been found extremely difficult or impossible to set up experimentally disease conditions similar to the natural disease. It would appear that certain conditions as yet unknown must be fulfilled before a natural case occurs. Features common to all these diseases are that the affected are generally in good condition and the history of a liberal supply of food being available is often forthcoming. Edgar (1927-28) showed the presence of anærobic spores in the livers of healthy sheep and any factor leading to diminished tissue oxygen may make the conditions suitable for the multiplications of such spores. It is possible that spores may lie dormant in tissues other than liver. Albiston (1937) describes cases of blackquarter in sheep due to the multiplication of *Cl. chauvœi* at the site of inoculation with *Cl. ovitoxicus* vaccine and suggests that the *Cl. chauvœi* spores were lying dormant in the tissues and that the inoculation of vaccine rendered conditions favourable. Conditions of reduced oxygen tension would be fulfilled by the setting up of a condition analogous to the Arthus phenomenon. Quick (loc. cit.) points out that the Arthus phenomenon can be reproduced experimentally in the lungs, kidneys, joints, heart, pericardium, brain, sub-mucosa of the stomach and the liver.

(*a*) *Braxy.*—In the case of this disease the conditions of occurrence are such as might favour the production of such a phenomenon. The occurrence of cases coincides with the presence of hoar frost and for this reason the condition has been attributed to the chilling effect on the stomach mucosa of a large quantity of grass covered with hoar frost so lowering the resistance as to allow the *Vibrion septique* to invade the tissues (Gaiger, 1922). However, even though a certain amount of inflammation were caused by such chilling, conditions will still not be suitable for *Vibrion septique. Vibrion septique* invasion is not a feature of gastro-intestinal inflammatory conditions of sheep other than braxy. A more

probable explanation of the association of hoar frost is that hoar frost being a large quantity of frozen moisture (McGowan, 1915) causes an excess of water in the rumen and this excess water will quickly begin to pass on into the other stomach compartments and into the intestine, carrying with it protein which will passing into the absorptive surfaces before it has had time to be properly broken down and is absorbed as foreign protein and shocks a sensitised animal. The factor of cold probably has an influence on the *locus* of the shock reaction. Auer (1919) showed that if in rabbits which had been sensitised with horse serum and then inoculated with sub-lethal doses of homologous antigen and 30 to 45 minutes afterwards had the ears treated with xylol, the irritating effect of the xylol appeared to facilitate the passage of antigen into the tissues of the ear and a local anaphylactic reaction with subsequent sloughing occurred. The irritating effect of the cold may also lead to the exit of absorbed foreign protein from the circulation into the tissues of the stomach mucosa, thus leading to a localised allergic reaction. Cold is recognised to be one of the causes of physical allergy (Swineford, 1935). In the case of a black frost excess water will not be ingested and thus there will be less likelihood of undigested protein being thrown on to the absorptive surfaces and it is well recognised that braxy does not occur under conditions of black frost. On this hypothesis four factors operate in the production of braxy (i) sensitised sheep, (ii) ingestion of food covered with large amount of frozen moisture, thus producing conditions favourable to absorption of the shocking dose of protein, (iii) the influence of cold determining the *locus* of allergic reaction, (iv) the presence of *Vibrion septique*.

(*b*) *Black Quarter.*—In this condition an Arthus-like phenomenon may occur in the muscles allowing *Cl. chauvœi* to multiply. A type of nodular necrosis called Roeckl's granuloma does occur in the muscles of cattle (Verge *et al.*, 1937, and Hole, 1938) and this may represent an Arthus lesion in which either the *Cl. chauvœi* was not present or the affected animal was previously immune.

(*c*) *Black Disease.*—This disease of sheep is attributed to the action of the immature liver fluke causing tissue damage and thus rendering conditions favourable for the multiplication of *Cl. chauvœi* (Turner, 1930). However, it is very rare for black disease to occur in a sheep heavily infested with liver fluke (Dodd, 1921), indicating that certain other factors may be necessary. That human beings may become sensitised to parasites has been shown by Rachemann and Stevens (1927) and bovines may be sensitised to hypoderma larvæ (Hadwen, 1922) so that allergic phenomena due to presence of liver fluke may be set up and pave the way for invasion by *Cl. œdematiens*. On the other hand, the migrating fluke may merely act as an irritant in the same way as cold does in braxy and determine the *locus* of an Arthus-like phenomenon.

(*d*) *Pulpy Kidney in Lambs and Enterotoxæmia in Sheep.*—These are generally associated with generous feeding, leading to infection with *Cl. welchii,* type D (Montgomerie and Rowlands, 1931; Oxer, 1932; Bennetts, 1932; Gill, 1933).

(*e*) *Lamb Dysentery.*—Hare and Glynn (1927) pointed out that the initial lesion in this disease is petechial and that bacterial infection is secondary to this. The present author's work (unpublished) on this condition, carried out while on the staff of the Animal Diseases Research Association, confirms these findings. Whether or not this increased capillary permeability is due to protein shock in lambs which have been sensitised *in utero* is not known.

In the prevention of these anærobic diseases by means other than immunological it appears that a lowering of condition is favourable. Bleeding as at time of castration and docking, periodic starvation and exercise are all considered to be means of cutting down losses. The value of the custom among flockmasters of dosing sheep with pig dung to prevent braxy may be due to its bringing about reduced body condition.

XI.—Diseases Characterised by Increased Vascular Permeability.

(*a*) *Lymphangitis in Horses.*—To quote Wallis Hoare (1913), "Heavy horses, well fed and accustomed to hard work furnish many instances of the affection when from any cause they are left idle for a varying period of time; but it is also met with in horses out of condition when they are suddenly changed in their surroundings and put on high feeding to which they are unaccustomed. Thus we meet with cases in country horses purchased for work in cities, when the animals are allowed full amounts of nitrogenous diet instead of the change being made a gradual one. There are many horses in which unlimited idleness and high feeding may not produce either of the affections. Horses subject to grease and chronic cracked heels are liable to attacks of lymphangitis if placed under circumstances favourable to the development of this affection. In horses that have once suffered from an attack, extra precautions are necessary."

Lymphangitis thus resembles some human allergic conditions in that it is a condition in which individuals among a group subjected to similar conditions show altered re-activity manifested by tissue œdema and that an animal once shown to be susceptible is liable to further attacks. One attack with proper treatment usually clears up without leaving much permanent tissue change but with repeated attacks permanent thickening of the affected limb occurs. Grease and chronic cracked heels are also probably manifestations of protein sensitivity and the tendency for multiple manifestations of allergy to occur in an individual is again a feature of human allergic conditions.

Davis (1932) records cases of lymphangitis in horses which received a liberal ration of freshly cut tares. Of 40 to 50 horses so fed five became affected.

(*b*) *Laminitis.*—Greaves (1855) writes of this condition as follows: "It appears to me that there must be, secretly and silently going on, a gradual diminution in the activity of the absorbents generally —an incomplete or defective performance of function in the capillaries, and the sentient fibres universally, causing a smothered or undeveloped irritability of the whole system, and which is only waiting, agreeably to nature's directions: and it is not until this igneous element has accumulated itself in sufficient quantities in the system, so as to acquire force, and to concentrate itself into a focus, that we are enabled to take cognizance of it; and even then it is not by any means clearly recognisable, until it is too evident that that focus is the laminæ, by its causing lameness." Such a conception entertained before protein hypersensitiveness was considered a cause of diease, is strongly suggestive of allergy. Greaves also stressed the fact that of a number of horses exposed to the same conditions only individuals were affected and that these same individuals had been subjected to similar conditions on previous occasions without harm ensuing.

Litt (1875) was of the opinion that there is a constitutional predisposition to this condition. Greaves (1864) speaks of a congenital tendency to the disease and Coe (1902) believes that the inheritance of a certain type of conformation predisposes to the condition. Williams (1864) and Woods (1889) do not believe that there is an hereditary tendency.

The disease has been observed to follow sudden changes in diet and overfeeding, Corby (1855), Williams (loc. cit.), Woods (loc. cit.), Thomson (1900), Coe (loc. cit.). Williams considers the condition due to an unhealthy state of a mucous membrane, generally that of the intestinal canal.

Penhale (1894), Thomson (loc. cit.) and Coe (loc. cit.) attribute cases of the disease to horses drinking flour or meal gruels between periods of work. Certainly such treatment must be a means of throwing undigested protein on to the intestinal mucosa as water is not retained in the horse's stomach and danger of shock will arise should the animal be sensitised. A factor in determining the laminæ as the shock organ probably is in some cases a degree of congestion due to concussion and contusion associated with hard work acting in the same manner as xylol on the skin in the experiment by Auer (*vide supra*).

(*c*) *Purpura Hæmorrhagica.*—Greig (1938) states, "The possibility that the disease is of anaphylactic origin cannot be disregarded. Such an origin is suggested by the experiment of Marek who injected a horse several times with streptococcal extract, with the result that after seven days typical purpura hæmorrhagica developed and terminated fatally." Ritzenthaler also regarded the disease as an

anaphylactic phenomenon as by repeated intravenous and subcutaneous injections of dilute white of egg he produced a similar form of disease and the active anaphylaxis so produced was transmitted passively to an untreated horse. Greig regards the disease as allied to the anaphylactoid urticarial purpura of man.

(*d*) *Diseases associated with Photosensitisation—Geeldikkop.*—This condition is met with (Theiler, 1918; Rimington and Quin, 1934; Quin and Rimington, 1935), among Merino sheep and Angora goats in parts of South Africa. The more hardy Afrikander sheep and Boer goat are not so susceptible. The disease appears to be most prevalent when a hot dry spell of weather sets in after a period of rain. Under these conditions the plant *Tribulus terrestris* grows quickly and then, with the onset of drought, wilts and it is the wilted plant which is said to be most dangerous. At other times this plant is a valuable food for these animals. Cases of the disease can occur in the absence of *Tribulus terrestris* and the ætiology of the condition cannot be claimed to be clearly established. Farmers also attribute the disease to the animals drinking water during the heat of the midday sun.

Those parts of the skin unprotected by wool become œdematous and later the skin over such areas becomes necrotic and sloughs. Marked jaundice is also a feature of the disease.

Rimington and Quin examined Tribulus plants for the presence of a fluorescent substance similar to the "hypericin" of St. John's wort with negative result. They, however, demonstrated the presence of phyllœrythrin, a porphyrin derived from chlorophyll, in the bile of affected sheep and were able to show that by inoculation of this material into healthy sheep, photosensitisation was set up. No jaundice developed in the experimental sheep.

This disease condition apparently makes its appearance when a supply of succulent food is made available to delicately constitutioned sheep and goats with soft white skins and when at the same time these are exposed to the hot sun. The conditions of occurrence resemble those of lactation tetany (*vide supra*) in that in both a generous supply of succulent or, in the case of the succulent food which has wilted, of easily disrupted, food is available. The factor of water supply probably also enters into both. In Geeldikkop the hot sun induces the animals to drink and in lactation tetany it is high-yielding cows which are most commonly affected (*vide supra*). In Geeldikkop, however, if this conception of the pathogenesis of the disease be correct, the irritating action of the hot sun on the delicate skin attracts the absorbed protein out of the circulation into the tissues and in sensitised animals an allergic reaction occurs in the skin tissue with consequent œdema. As pointed out above, in braxy it may be the action of cold which determines the *locus* of the shock reaction and in this condition it is the action of the sun's rays. Both of these physical agents are recognised to be factors in the production of cases of so-called physical allergy in the human subject (Swineford,

loc. cit.). Cohen (1935) and Piness (1935) found that by elimination of sensitising foreign protein such cases no longer occurred on re-exposure to the physical agent.

Lamont (1938) describes an outbreak of disease in which five valuable Border Leicester sheep being prepared for show and receiving a liberal concentrated ration composed of special sheep nuts with 2 per cent. of an iodised mineral mixture which contained cod liver oil and malt, showed œdema of the face and ears and died. Other sheep receiving the same foodstuff but in a less amount did not develop the disease. Lamont suspected the disease to be a photosensitisation associated with the ingestion of some plant or protein and regarded the condition as similar to fagopyrism associated with the ingestion of buckwheat and to Geeldikkop. Since these cases occurred in Ireland from the end of February to about the middle of May, it must be assumed that the animals were extremely sensitive to sunlight if photosensitisation were a factor, as the distribution of the lesions indicates. This condition may be comparable to those cases of laminitis occurring in stallions being prepared for shows (Woods, loc. cit.).

(*e*) *Angio-neurotic Oedema? of the Pig.*—Shanks (1938) drew attention to a condition met with in young pigs when these are given a ration of rich concentrated food without an intermediate period of moderately rich food. The best conditioned pigs are first affected. A weakly one generally escapes. Sudden fatal illness is a feature of the condition. On *post-mortem* examination œdema of the eyelids, œdema of the wall of the stomach or of the mesentery between the folds of the large intestine are the principal lesions. Sometimes part of the small intestine shows a hosepipe appearance. Such tissue changes resemble those described in human angio-neurotic œdema with gastro-intestinal manifestations though sudden death is not a feature of the latter condition.

(*f*) *Angio-Neurotic Oedema of the Dog.*—Phillips (1922) records two cases of this condition in highly bred dogs, one on being fed a large meal of ham and the other on being fed fresh or canned fish. The first was kept on pork-free diet and no further attacks occurred. A year after the attack skin test gave a positive reation to pork. There was no ancestral history of cases and the progeny of the case was normal. In the second case attacks occurred on eating fish and when this was withheld attacks ceased. No skin tests were made. Five years' abstinence from fish appeared to lead to spontaneous desensitisation as the dog could then eat fish with impunity. No similar cases were known among the ancestors but one of the offspring died of eclampsia and one puppy suffered from asthma and later from erythema multiforme.

Schnelle (1933) states that angio-neurotic œdema of dogs occurs comparatively frequently, symptoms being œdema of the face, cheeks, over eyes and sometimes other parts of the body. He finds it most common in the young and in many cases the first time

lamb is fed. It seldom recurs, indicating an increased tolerance or its elimination from the diet.

(*g*) *Rosenfeber.*—This condition is attributed to anaphylaxis due to protein absorbed from hypodermal larvæ should these be ruptured *in situ* in the subcutaneous tissue of the back of cattle. In the more benign types œdematous swellings occur in various parts of the body. On the other hand, on occasion, death may occur in a few minutes (Hadwen, 1922).

XII.—Skin Diseases.

(*a*) *Eczema in Dogs.*—Schnelle (loc. cit.) considers this to be an allergic condition and carried out skin tests on two eczematous dogs. Both dogs were found sensitive to salmon and other foods and by feeding salmon and potatoes a recurrence of eczema was produced. Burns (1933) and Pomeroy (1934) carried out extensive skin tests on dogs and positive reactions to foods were obtained in diseased and also in normal dogs. Some feeding tests were also carried out. The results appear rather inconclusive and point to the inherent difficulties of the whole problem.

(*b*) *Potato Dermatitis.*—This is probably allergic in origin. It occurs principally in fattening stables of potato-spirit distillers in oxen or dry cows fed for prolonged periods on potatoes or their waste products especially distillery waste. It appears that an idiosyncrasy exists especially as some animals are attacked after having taken only a few bucketfuls of the liquid while others can take large quantities over long periods without ill effects (Johne quoted by Greig, 1938). Greig also states that " the frequency with which the eruption is confined to the feet is explained by the fact that these parts of the body are particularly exposed to mechanical irritation, soiling with excreta, damp, etc., and are thus specially susceptible to the potato toxin. The comparative immunity of milch cows and the occurrence of the disease or of acute gastro-intestinal catarrh in the calves and foals fed with the milk of such cows can both be explained by assuming that the toxin is excreted in the milk." Shannon (1921) found that food proteins could pass into the milk. Thus food protein sensitisation may cause such conditions both in the cattle and their offspring.

(*c*) *Grease in Horses.*—This condition affects the skin of the distal parts of the limbs, more often the posterior limbs. Möller (1890) considers that cold has an unfavourable effect in setting up the disease. He also quotes Foillet who attributed the disease to cold weather and icy, dirty water. Hereditary predisposition is considered to be a factor in determining the occurrence of the disease.

XIII.—Drug Allergy.

Quick (loc. cit.) postulates that since cincophen can produce other known allergic reactions, the yellow liver atrophy or necrosis which it produced may be a manifestation of the Arthus phenomenon in

the liver especially since many of the cases showed a previous allergic hypersensitivity. Particularly significant is the fact that severe hepatic damage resulted from relatively small doses whereas large doses over long periods in non-sensitive patients apparently produce little if any liver change. He recommends that cincophen and similar drugs should not be given simultaneously with foreign proteins (vaccines, milk parenterally).

Hudson (1932) records heavy losses in ewes dosed with carbon tetrachloride. These ewes were dosed three times with first interval of a month and second of several weeks. They are described as showing symptoms of acute jaundice. This mortality was possibly due to drug allergy.

In veterinary medicine it is recognised that dosage with carbon tetrachloride of sheep receiving a ration of concentrates is more dangerous than similar dosage of sheep receiving only natural food (Montgomerie, 1927; Norris, 1927; Spicer, 1927; Bowes, 1932; Rose, 1932). This susceptibility of sheep receiving concentrates may have a bearing on the whole problem of allergy in the domesticated animals and may indicate that foreign protein tends to be absorbed when animals are receiving concentrates and that such protein combines with the carbon tetrachloride. It is doubtful whether these deaths are the result of drug allergy. Montgomerie (personal communication) recalls two cases of poisoning with carbon tetrachloride in which there was a definite history that in neither case had the sheep or their parents been previously doses with this drug. He also finds that sheep can be dosed two or three times each year for several seasons without the appearance at any time of symptoms of poisoning.

Bacterial Allergy.—Owing to the great extent of the scope of this important type of allergy it has not been dealt with in this, more general, paper.

XIV.—General Discussion.

In this paper a number of diseases met with among the domestic animals have been collated and evidence has been submitted that sensitisation to food protein, to tissue protein or to metazoon parasite protein may be common to all of these conditions.

An examination of the various diseases from this aspect points to a number of factors which contribute in the production of this state of hypersensitiveness. Generous feeding appears to be one of the most outstanding ætiological factors. This may act in two ways (i) in bringing about changes in the body tissues which favour sensitisation, (ii) by leading to the absorption of food protein in its native state. Regarding tissue changes, there is often the history of the affected animal being in good condition or in a rapidly thriving condition at the time of onset. In the early stages of the process of fattening of animals, the rate of weight increase is much greater than in the later stages. This is probably due to accumulation of water in the tissues and not solely due to deposition of fat.

As has been pointed out above, in epilepsy there is a tendency for water storage to occur and this leads to periods of seizures. In cases of human asthma, Sheldon *et al.* (1939), has shown that during prolonged attacks there is increased urinary water loss. This probably indicates that the action of a protective mechanism on the part of the body, to rid its tissues of excess water, comes into play during attacks. The value of purgatives administered immediately before and after parturition in preventing the occurrence of milk fever may be related to the dehydration effect on the tissues. This may also be the explanation of the beneficial effect attributed to periodical purgation in the prevention of recurrences of lymphangitis. The value of exercise in preventing lymphangitis and enterotoxæmia and the value of a difficult parturition in preventing milk fever may also be related to a dehydrating effect. The value of bleeding in the prevention of pulpy kidney may also be due to the dehydrating action.

It might be postulated that the benefits of purgation are due to the removal of toxic substances from the alimentary canal, especially as some disease conditions, such as lymphangitis and laminitis, often have a history of overfeeding or irregular feeding but of the presence of such a toxin there is no direct evidence except in the case of certain diseases associated with the multiplication of anærobic bacteria in the alimentary canal and in these cases it is probable that the alimentary canal is itself the *locus* of a shock reaction and that the multiplication of anærobes is secondary. The peculiar localisation of some of the diseases dealt with does not indicate a toxæmia. In a toxæmia one would expect that all the predilection tissue would be affected to some extent in an acute attack whereas, here, the lymphatics of only one limb or the laminæ of one foot (more than one is often affected but not necessarily so) are the sites of tissue change.

In many of the conditions under discussion there is often a definite history of irregular or over-feeding and if the diet can be and is carefully controlled these cases do not occur. This would appear to indicate that the domestic animals are not easily shocked and that it is only under conditions which lead to an excessive flooding of the intestine of a sensitised animal with native protein that cases occur. It is obvious that human and economic factors will continue to play an important rôle in the production of many of these diseases. In the case of dog hysteria, our knowledge of the conditions of occurrence is not well defined and although changes in diet have often been claimed to cut down the incidence of the disease, the exact reason why improvement was obtained is not established and we are not able to control the disease to the same extent as we can some of the others.

In the acute conditions under review there is no very definite history of hereditary factors coming into operation though occasionally a familiar tendency is recorded. Regarding the chronic diseases

of the nervous system, on the other hand, most investigators are of the opinion that heredity has a very marked influence on the occurrence of these diseases and in this respect they are similar to the human allergic diseases. They do not, however, resemble human allergies in all respects: there is no evidence of intermittency even in the earlier stages though it is possible that animals are not sufficiently closely observed. In fowl paralysis the cellular infiltration into the nerve trunks renders it improbable that any intermittency occurs.

In a number of the conditions under discussion tissue irritation appears to play an important part in their causation. Such irritants are the rays of the sun in photosensitisation diseases, the action of cold in braxy, the soiling of the skin with excreta in potato dermatitis, the action of cold and wet in grease, the action of concussion and contusion in laminitis. These disease conditions do not occur in all animals exposed to such agencies and the now affected animals may have been similarly exposed on previous occasions without apparent harmful effects. It would appear that the animal must be in a state of sensitivity to some other additional factor. This other factor has been said to be photosensitising pigments or toxic metabolites. Also under conditions of domestication animals are called upon to produce in greater abundance than under natural conditions. In order to do so it is essential that these animals consume more food and water than the low producer and it is possible that under certain conditions the body fails to deal satisfactorily with the added load and disease occurs. In the high producing milk cow the endocrine glands have been postulated as being the weak link when diseases such as milk fever and lactation tetany occur. At attempt has been made in this communication to show that all these conditions are more likely to be different manifestations of the action of one ætiological agent, namely, foreign protein, and that conditions of domestication, by demanding that the animals produce more, also requires them to eat and drink more and often physiologically irregularly, and also food of a kind for which the alimentary canal is not suited is often fed, all of which may lead to the absorption of food protein in its native condition.

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A *SALMONELLA* STRAIN ISOLATED FROM CAMELS IN PALESTINE.

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In October, 1939, the organs of a camel suffering from enteric symptoms and cachexia were subjected to a bacteriological examination. From the ileum, kidneys and heart, bacteria were isolated which fermented glucose, maltose, mannose and arabinose with the production of acid and gas; tartrate and citrate were attacked and on Kligler medium hydrogen sulphide was formed. Lactose and sucrose were not attacked. The organisms were agglutinated by an *S. newport* serum (O) and by an *S. typhi-murium* serum (H-agglutination). Together with another strain isolated from the intestines of a second camel suffering from the same symptoms, they were sent to the *Salmonella* centre for Palestine (Professor A. Klopstock in Tel Aviv) for further identification. Professor Klopstock established the serological identity of these organisms with *S. kentucky* with the antigenic formula VIII XX:i-z6-. Experiments showed the complete serological identity of the O-antigens of the camel strains and the *S. kentucky* strain. Two sera prepared by immunisation of rabbits with bacteria heated at 100° C. for two hours were used. Absorption of the *S. kentucky* serum (O) as well as of the camel-strain serum (O) with *S. newport* left agglutinins for both the Kentucky and the camel strains. Absorptions of these sera with *S. kentucky* or with one of the camel strains freed the serum from all somatic agglutinins for all camel strains and for *S. kentucky*.

Similar experiments were made with two sera prepared by immunisation with bacteria heated at 60° C. for 30 minutes. By absorption with bacteria heated at 100° C. the somatic agglutinins were removed and the following experiments undertaken with the flagellar agglutinins. Absorption of the camel-strain serum with *S. kentucky* removed the agglutinins for all strains of the group; absorption of the *S. kentucky* serum with most of the camel strains removed the H-agglutinins for all the camel strains as well as for *S. kentucky*. One of the camel strains left a well-defined residue of H-agglutinins in the *S. kentucky* as well as in the camel-strain serum but freed the serum of all its own agglutinins. The agglutination of this strain by a specific *S. typhi-murium* serum, containing the i-agglutinin, and by an *S. kentucky* serum absorbed with *S. typhi-murium* and containing only the z6-factor, showed that this strain contained only the i-factor representing the alpha-phase of the organism as described by Ph. R. Edwards (1938).

The results of the bacteriological and serological examination of a further group of camels are shown in Table I.

TABLE I.

RESULTS OF THE EXAMINATION OF 19 CAMELS FROM DIFFERENT PLACES.

No.	*Place.*	*Age in Years.*	*Findings in Camels.*	*Bacteriological Examination.*	*Titres of agglutination with the Camel Strains and with S. kentucky.*
1	Trans-jordan	More than 12	Normal	Negative	1 : 100 (O)
2	Gaza	„ „ 12	Cachexia	„	1 : 100 (O)
3	„	„ „ 12	Normal	„	1 : 200 (H and O)
4	Hebron	6	„	„	1 : 50 (O)
5	„	10	Cachexia, 5 litre peritoneal fluid	„	1 : 50 (O)
6	„	3	Normal	„	1 : 50 (O)
7	„	8	8 litre peritoneal fluid Diarrhoea, Echinococcus in liver and lungs	„	1 : 10 (O)
8	„	7	Normal	„	1 : 50 (H and O)
9	„	10	„	„	1 : 50 (O)
10	Lydda	12	„	„	1 : 50 (O)
11	„	10	„	„	1 : 200 (H and O) 1 : 500—1 : 1000 (H)
12	Gaza	6	„	„	1 : 100 (H and O)
13	„	8	„	„	1 : 100 (H and O)
14	Hebron	8	Cachexia, 5 litre peritoneal fluid	*Salmonella* (camel-strain) in blood culture	1 : 500 (H and O)
15	„	8	Cachexia, Diarrhoea 6 litre peritoneal fluid	Negative	1 : 100 (O)
16	Lydda	More than 12	Normal	„	1 : 200 (O)
17	„	10	„	„	1 : 200 (H and O)
18	„	12	„	„	1 : 500 (H and O)
19	„	More than 12	„	„	1 : 200 (H and O)

The table shows that the serum of healthy as well as of infected camels contains O- and sometimes H- and O-agglutinins for the camel strains and for *S. kentucky*. The results with the camel strains as well as with *S. kentucky* were quite uniform. Absorption tests showed that *S. kentucky* as well as any one of the camel strains remove the agglutinins for all the strains of the group.

The strain isolated from the heart blood of camel No. 14 was serologically identical with *S. kentucky* and the camel strains previously isolated. Of the five camels with cachexia and enteric symptoms four were from Hebron and one from Gaza. Up to the present there were no cases reported from other parts of the country.

Table I further shows that the sick camels have a relatively low agglutination titre. From five cachectic animals only one titre of 1 : 200 and more, two of 1 : 100, one of 1 : 50 and one of 1 : 10 were obtained. The camel strain was isolated from one of these cachectic camels. On the other hand the serum of most of the

healthy camels showed agglutination titres of 1 : 100 and more. Four camel sera showing the presence of H- and O-agglutinins for *S. kentucky* were tested with six other *Salmonella* strains which have no common H-antigens with *S. kentucky* and the camel strains. The results are tabulated in Table II.

TABLE II.

AGGLUTINATION TITRES OF 4 CAMEL SERA FOR VARIOUS *Salmonella* STRAINS.

Strain.	*Serum No.* 11.	*Serum No.* 14.	*Serum No.* 18.	*Serum No.* 19.
S. kentucky	1 : 500 (H and O)	1 : 500 (H and O)	1 : 500 (H and O)	1 : 200 (H and O)
Camel-strain	1 : 500 (H and O)	1 : 500 (H and O)	1 : 500 (H and O)	1 : 500 (H and O)
S. paratyphi A	Negative (at 1 : 50)	Negative	Negative	Negative
S. paratyphi B	1 : 100 (O)	1 : 200 (O)	1 : 200 (O)	1 : 100 (O)
S. paratyphi C	1 : 200 (O)	1 : 100 (O)	1 : 100 (O)	1 : 100 (O)
S. enteritidis	Negative	Negative	1 : 50 (O)	1 : 50 (O)
S. typhi (H901)	„	1 : 100 (O)	1 : 200 (O)	1 : 100 (O)
S. london	„	Negative	1 : 50 (O)	1 : 50 (O)

The pathogenity of the camel strains and of *S. kentucky* was tested by oral infection of mice. The animals were observed for a period of 20 days. The results were recorded by noting the specific mortality, based on cultures from the dead mice; and at the end of each experiment, by the residual infection determined by culturing the heart, liver, spleen, kidneys and mesenteric glands of the surviving mice.

After an infection with 1,000 millions of bacteria, the following results were observed:—

Camel Strain.—One death, blood culture positive; nine mice dissected showed all organs and the mesenteric glands sterile.

S. kentucky.—No death. Two mice dissected showed infections of liver and spleen, three infections of the mesenteric glands and five were sterile.

Another group of mice received the same dose on the two following days so that a total amount of 2,000 millions of bacteria was given. The results were as follows:—

Camel Strain.—No deaths. Two mice showed infections of the mesenteric glands, six mice were sterile.

S. kentucky.—No deaths. One mouse showed a general septicæmia, three mice showed infections of the mesenteric glands, four mice were sterile.

A third group received the same dose on four consecutive days. The results were as follows:—

Camel Strain.—No deaths. One mouse dissected showed a general septicæmia, one mouse an infection of the abdominal organs, six mice were sterile.

S. kentucky.—Three deaths, the heart blood was positive. One mouse dissected showed an infection of the abdominal organs, one

mouse had infected mesenteric glands and three mice were sterile.

The experiments show that the camel strain and *S. kentucky* have a low pathogenicity for mice, the mortality is low and the infection is either localised mainly in the mesenteric glands or the animal is sterile. But it seems that *S. kentucky* has a tendency to persist for a longer period in the liver, spleen and mesenteric glands.

The general results of these three experiments were as follows:—

	Camel strain.	*S. kentucky.*
Total infected	26	26
Dead	1	3
Survived and dissected:—		
Septicæmia	1	1
Abdominal organs infected	1	3
Localised infection of mesenteric glands	2	7
Sterile	21	12

The strains isolated from the mice showed in the first generation a partial or total loss of the H-agglutinins. They appeared as O-forms with a total loss of the H-antigens, or they contained only the i-antigen representing the *alpha* phase of the strain. After two to three passages they again showed full agglutinability for the i- and for the z6-agglutinin.

Experiments were now undertaken with strains isolated from infected mice. After feeding of 4,000 millions of bacteria the results were as follows:—

	Camel strain.	*S. kentucky.*
Total infected	14	14
Dead	0	0
Septicæmia	0	0
Abdominal organs infected	3	1
Localised infection of mesenteric glands	3	2
Sterile	8	11

The camel strain after a passage through mice showed a somewhat higher number of residual infections than formerly, but the mortality was zero.

In the following experiments we compared the fermenting activities of *S. kentucky* and the camel strains towards different carbohydrates. Both strains fermented the following carbohydrates: arabinose, xylose, fructose, glucose, galactose, mannose, maltose, melibiose, dulcitol, sorbitol, trehalose; they did not attack sucrose, lactose, melizitose, raffinose, alpha-methylglycoside, starch, inulin, glycogen.

S. kentucky fermented glycerol and inositol in plain broth, but the camel strains did not attack them.

Summary.

1. Experiments are reported demonstrating that there exists in Palestine an infection of camels with a *Salmonella* strain. This strain was isolated from the blood and organs of three camels showing enteric symptoms and cachexia.

2. The camel strains showed the serological structure of *S. kentucky* but they did not attack glycerol and inositol in plain broth.

3. From 19 sera of camels, nine contained H- and O-agglutinins for *S. kentucky* and the camel strain, the 10 other sera only O-agglutinins.

4. Four camel sera which contained H- and O-agglutinins for *S. kentucky* and the camel strain contained only O-agglutinins for other *Salmonella* strains.

5. Infection of mice *per os* with both strains gave a low mortality, but residual infections of the abdominal organs and the mesenteric glands varying from about 20 to 40 per cent. of the infected animals were found three weeks after the infection.

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SALMONELLA INFECTIONS OF DUCKS AND DUCKLINGS

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Introduction.

The occurrence in domesticated birds of epizoötic disease caused by infection with members of the Salmonella group other than *S. pullorum* and *S. gallinarum*, is not uncommon, and has been frequently recorded during the past 20 years. For clarity the references on this subject can best be shown as in Table I, which indicates the organism identified and the species of domesticated birds from which these members of the Salmonella group have been isolated.

Table I.

Organism.	*Species.*									
	Chicks.		*Ducks and Ducklings.*		*Turkeys and Poults.*	*Pigeons.*	*Pheasants.*	*Goslings.*	*Canaries.*	*Parrots.*
Salmonella typhi-murium	(3) (12) (16) (21)		(2) (7) (10)	(5) (8) (21)	(17) (18)	(13) (15) (19)	(21)	(21)	(14)	(13)
S. enteritidis Gærtner	(16) (21)		(5) (10) (20)	(9) (16) (21)	—	—	—	—	—	—
S. anatum	(12)		(1) (4)	(2)	—	—	—	—	—	—

(The figures in brackets denote the number of the reference in the bibliography.)

In the course of our routine laboratory work infection with *S. typhi-murium*, and *S. enteritidis gærtner* has been diagnosed on many occasions. From 1933 to 1939 *S. typhi-murium* was isolated in 57 instances and *S. enteritidis gærtner* in 8, and the species involved are illustrated in Table II.

Table II.

	Chicks.	*Ducklings.*	*Pheasant Chicks.*	*Turkey Poults.*	*Goslings.*	*Pigeons.*
S. typhi-murium ...	32	13	3	4	1	2
S. enteritidis Gærtner ...	3	4	—	—	—	—

In addition *S. typhi-murium* was recovered from one adult duck (ornamental type and a single death) and from an outbreak in guinea-pigs, while *S. enteritidis gærtner* was also recovered from an adult turkey.

Although from Table II it will be observed that these organisms have been isolated with more frequency from chicks, the most extensive outbreaks involving the largest number of losses have been encountered amongst ducklings, and it is with the observed manifestations of Salmonella infection in ducklings that this report is concerned.

It is a fair assumption, borne out by these observations, that the increase of the disease in ducklings is associated with the adoption of intensive methods of management on duck farms, and there is a possibility that Salmonellosis may assume the same economic importance in the rearing of ducklings as Pullorum disease in the rearing of chicks.

The recorded instances of Salmonella infections in ducklings, involving heavy losses, commence with the epidemic recorded by Rettger and Scoville (1920) in America. These authors described a disease which was termed "Keel" and which caused losses amongst thousands of ducklings, approximating 90 per cent. of those hatched. The Salmonella organism isolated from the affected ducklings was considered by the authors to show distinctive features and was named by them *S. anatum.* Later, Edwards and Rettger (1927) recorded that they had re-examined the strains of organisms isolated from these ducklings, and found them to be divisible into two types, one identical with *S. typhi-murium,* and the other a new species for which the name *S. anatum* was retained. They further record three large outbreaks of "Keel" disease, from which ten strains of Salmonella organisms were isolated and studied. Serologically seven strains were found to be identical with *S. typhi-murium,* and three with *S. anatum.*

In this country, Doyle in 1927 recorded an outbreak of disease in young chicks, with a mortality of 100 per cent., from which *S. typhi-murium* was isolated. Simultaneously, on the same farm, 69 per cent. deaths occurred amongst the ducklings, and although no specimens were received for examination the owner considered them to have been affected with the same disease.

Later, also in England, Gaiger and Davies (1930) recorded a severe epidemic of "Keel" disease, involving the loss of 4,000 ducklings during one year. As the result of their examinations they were able to demonstrate that the infective agent was a Salmonella organism, considered by them to be identical with *S. anatum* described by Rettger and Scoville, but later workers—Hole (1932) and Lovell (1932)—re-examining the organism, came to the conclusion that it was, in fact, *S. enteritidis gærtner.* Hole also recorded three further epizoötics in ducklings, from which he isolated an

organism identical with *S. typhi-murium* in two instances, and *S. enteritidis* in the other.

The disease has also been identified in South Africa; in 1934 Dunning described an outbreak of "Keel" in ducklings in which a Salmonella organism, closely resembling *S. enteritidis gærtner,* was found.

History of Outbreaks under Observation.

During the winter, spring and early summer of 1938-39 an opportunity was presented of investigating an extensive outbreak of Salmonellosis involving thousands of ducklings. In addition to the laboratory work, it was possible to keep fairly closely in touch with the farms involved, and to follow the results of the preventive and control measures adopted, both in the ducklings and adult breeding stock.

In the first instance, ducklings were received at the laboratory with the information that large numbers were dying, and that for some time past the mortality rate had been well above normal. *Post-mortem* examination revealed no specific lesions, and on bacteriological investigation *S. typhi-murium* was isolated from the tissues of each of the ducklings. Further specimens were obtained and again *S. typhi-murium* was isolated and was assumed to be the cause of the disease.

On visiting the infected premises it was found that the farm was concerned solely with the rearing of ducklings from day old to 12 weeks old, for distribution to fatteners. There were approximately 57,000 ducklings on the premises, 45,000 from day old to four weeks old in brooder houses, and 15,000 from four to nine weeks old on free range.

In the brooder houses the losses up to four weeks totalled 30 per cent. Some batches showed considerably higher mortality than others, but over all groups the losses amounted to more than 1,000 ducklings per day. Losses were negligible once the ducklings had left the brooder houses, and on range little mortality occurred.

The course of the disease in each group showed a constant feature, in that two peak periods of mortality were experienced. The first occurred from three to four days after hatching, when numerous deaths were recorded, followed by a period of low mortality to the seventh day, after which deaths increased rapidly to a second and higher level at about 12 to 14 days, and thereafter declined until, on removal of the ducklings from the brooder houses at the 28th day, losses had almost ceased.

Symptoms.

Ducklings dying up to the third or fourth day after hatching showed no definite symptoms and were usually found dead. After seven days, when the major losses occurred, constant symptoms were

observed, taking the form of sluggishness, inco-ordination of movement, staggering, lameness or weakness of one or both legs, and drawing back the head until the beak rested dorsally on the spine, with the lower mandible uppermost. Sometimes circus movements, as falling over backwards or on the side, kicking violently, and twisting of the head in circles, were seen. These symptoms were often intermittent, two or three attacks occurring at intervals throughout the day, and death finally taking place in one of the spasms, usually within 24 hours of the onset of symptoms. Thirst was increased, and it was not uncommon for apparently healthy ducklings to leave the drinking fountains after drinking, exhibit one or other of the above symptoms and die within a few minutes. A greenish diarrhœa was fairly constantly present.

These symptoms show a marked similarity to those quoted by Rettger and Scoville and by Gaiger and Davies in the outbreaks of "Keel" disease recorded by them in U.S.A. and Great Britain respectively. Certain of these symptoms, particularly the age susceptibility, the occurrence of sudden death after drinking, and staggering, have been observed in many of the outbreaks previously recorded in the laboratory. We have also been informed by duck farmers and others closely connected with the duck industry that similar symptoms have been recognised amongst young ducklings for many years and described variously by them as apoplexy, sunstroke, cerebro-spinal meningitis, staggers. It therefore seems possible that many of the these outbreaks may have been due to Salmonella infection.

Post-mortem Lesions.

In most cases *post-mortem* lesions were not constant or characteristic, and were of little aid to diagnosis. In ducklings from one to ten days of age the only lesions occurring with any regularity were marked congestion of the lungs and retention of the yolk sac. These lesions could indicate any non-specific disease associated with mismanagement and, as with Pullorum disease in chicks, diagnosis could only be made after bacteriological examination.

In the older ducklings, 10 to 21 days of age, more definite lesions were observed *post-mortem*. The most constant lesion was a mottled appearance of the liver, due to irregular areas of congestion, the organ showing slight enlargement. In a few specimens the liver capsule was raised by a gelatinous exudate and was easily detachable, while in three instances rupture of the liver, with internal hæmorrhage, was noted. In many of the ducklings examined, the kidneys were pale and showed bright-red hæmorrhagic streaks. As in the younger ducklings, congestion of the lungs was almost constant. In one or two instances a sero-fibrinous pericarditis was observed.

Bacteriological Examination.

Representative specimens of affected ducklings from the various age groups, including ducklings reared in batteries to 12 days old on

the breeding farm and subsequently transferred to the rearing farm, were obtained for examination, and the results are shown in Table III.

TABLE III.

Group.	*Age.*	*Number Examined.*	*Results.*
1	Day old	10	*S. typhi-murium* recovered
2	3 days old	10	*S. typhi-murium* recovered
3	10 days old	6	*S. typhi-murium* recovered
4	12 days old reared in batteries from day old	3	No organism recovered
5	15 days old	9	*S. typhi-murium* and *S. enteritidis* Gaertner recovered
6	21 days old	3	No organism recovered
7	22 days old	5	*S. typhi-murium* recovered
8	Indeterminate	5	*S. typhi-murium* and *S. enteritidis* Gaertner recovered

Note.—Groups 4 and 6, the only groups from which the organisms were not recovered, were reared from the same stock on separate premises, and did not pass through the brooder houses on the rearing farm.

The ducklings were examined bacteriologically by the inoculation of material from the liver, and pericardial fluid if present, into "Lemco" broth and on to nutrient agar slopes. Primary cultures were found to grow readily and in almost every instance were found to be pure, no selective media being required. Identification was carried out by staining smears by Gram's method to note purity, followed by inoculation into carbohydrate media. For routine examination purposes, only three carbohydrates, lactose, maltose, and dulcite, were used. Broth cultures were then submitted to agglutination tests against known positive *S. typhi-murium* and *S. enteritidis gærtner* sera.

No differentiation could be made between *S. typhi-murium* and *S. enteritidis gærtner* on primary culture. Both grew readily on ordinary media, showing a uniform turbidity in broth, much heavier than the growth of *S. pullorum,* while on agar slopes a heavy, viscous, confluent growth was obtained. All cultures were found to be Gram-negative, non-lactose-fermenting bacilli, forming acid and gas in maltose and dulcite. Differentiation was based on the results of the agglutination tests with known sera. In general *S. enteritidis gærtner* fermented dulcite more slowly than *S. typhi-murium,* and required several days' incubation for fermentation to occur. At a later date representative cultures of pure strains from the various groups of ducklings were studied in greater detail, and were found to be typical *S. typhi-murium* and *S. enteritidis gærtner* in their cultural characteristics. This work is not yet complete and a full detailed report will be given later.

At the same time six representative cultures of each organism were submitted to Dr. W. M. Scott of the Ministry of Health's

Pathological laboratory, who kindly examined their antigenic structure and reported (private communication) that six were typical *S. enteritidis gærtner* and six typical *S. typhi-murium.*

Control Measures.

Following on the laboratory diagnosis of the outbreak it was necessary to outline measures of control, and these divided themselves under two heads.

1. Control of the infection amongst the ducklings on the rearing farm, and
2. Detection of possible carriers in the breeding stock.

1. *The Rearing Farm.*

A visit was paid to the rearing farm and a careful investigation made of the management and hygiene, and in view of the importance which was subsequently attached to this aspect of the outbreak, the conditions are described in detail. The ducklings were hatched on separate premises and brought to the rearing farm as "day olds," and there reared in brooder houses for four weeks, when they were transferred to free range. The ducklings were received as "day olds" from the hatchery in consignments of 2,500 to 3,000 on four days of each week, thus ensuring constant and continuous movement of ducklings through the brooder houses. The brooding methods and general management followed normal lines, except for the very large numbers involved.

The ducklings were housed in wooden brooder houses, 300 feet by 30 feet with hot-water pipe hovers, ridge ventilation and hopper type windows, and with water laid on to drinking fountains in each compartment. Each brooder house had a food store attached, in which the food was mixed and distributed three times daily in shallow, movable feeding-troughs. The food stores and brooder houses were not vermin proof, and signs of rats and mice were evident in both. Peat moss litter was used, and when compartments were cleaned out, the litter was thrown out through the windows to the ground outside, where it remained until the opportunity occurred to remove it, which might be after an interval of several months. The drainage of both land and houses was very poor, and water had been known to flood the lower portion of the houses.

Hygiene generally was of a low standard, and in addition to the practices mentioned it was observed that shovels used for cleaning out the litter were also used for mixing the food, and no attempt was made to keep vermin from the foodstores or houses. The continuous movement of ducklings throughout the houses was such that no section was vacant long enough to allow for efficient cleansing to be carried out. The cleansing of food and water troughs was very irregular and inefficient. The boxes used for conveying ducklings from the hatchery to the rearing farm were used until so

dirty as to make further use impossible. In general, the ducklings were overcrowded.

It was pointed out to the owners that, having allowed an infectious disease to establish itself unchecked amongst such large numbers of ducklings, the problem of its eradication had been rendered extremely difficult, if not insoluble, and that only drastic measures of improvement offered any hope of success.

The major alterations which we considered necessary, and outlined to the owners, were:—

1. Each house to be completely emptied of ducklings for a sufficient period to allow of thorough disinfection.
2. The partitioning of houses to form smaller self-contained units.
3. The number of day-old ducklings arriving from the hatchery to be regulated to prevent overcrowding, so that two or three units were always vacant for a period to allow of disinfection between batches.
4. An efficient drainage system of houses and land.
5. Hygienic and more frequent removal of litter from the houses to a separate manure heap.
6. The provision of a separate vermin-proof food store, with separate personnel.
7. Daily cleansing of food and water troughs.
8. Non-returnable boxes, destroyed after use, to be used for the transport of the day-old ducklings.

As was surmised, all the improvement suggested could not be carried out, particularly the structural alterations, but the main recommendations for the improvement of general hygiene were adopted. The results were most successful, for the mortality in the brooder houses amongst ducklings up to four weeks of age fell from 30 per cent. to under 3 per cent.

2. *The Breeding Flock.*

In view of the references in the literature to the isolation of these organisms from duck eggs Scott (1930 and 1932), Warrack and Dalling (1932 and 1933), Beller and Reinhardt (1935) and the detection of adult carriers by means of the agglutination test, Scott, Warrack and Dalling, together with the indications in various outbreaks observed that infection had been transmitted *via* the egg, it was considered that eradiation could not be effective if adult carriers were allowed to remain in the breeding flocks.

Accordingly arrangements were made to test the breeding flock both for *S. typhi-murium* and *S. enteritidis gærtner* infection. The results of the testing of the breeding flock are shown in Table IV. There was a period of about two to three months between each test.

Table IV.

Test.	*Approximate Number of Samples Tested.*	*Percentage Number of Positive Reactors.*
1st	21,000	4·6
2nd	18,500	5·9
3rd	10,400	4·4

The positive reactors were in the proportion of one reactor to *S. typhi-murium* to four reactors to *S. enteritidis gærtner,* and it was found that the percentage number of reactors increased with the age of the birds. The distribution of the carriers at the first test throughout the various age groups is shown in Table V.

Table V.

Year Hatched.	*Number Tested.*	*Number of Reactors.*	*Percentage.*
1935	173	30	17·3
1936	4,050	400	9·8
1937	7,750	367	4·8
1938	7,150	100	1·3

It will be obvious that the testing was not successful in reducing the percentage number of carrier birds. On a subsequent visit to the breeding farm it was learned that this could be due to two factors. In the first instance non-reactors had not been removed to fresh ground, and no attempt had been made to carry out disinfection of houses or utensils after the removal of reactor birds, and in general the conditions on the farm favoured the persistence of infection. Secondly, there was known to be a considerable population of rats and mice and sporadic efforts to exterminate them had been ineffective. As these vermin are known to be susceptible to natural infection with these organisms it is highly probable that they served as a reservoir of infection, which may have been transmitted to the ducks by the continued contamination of food, water, or litter by infected excreta.

Summarising the control measures, it must be obvious that the most effective method of reducing the gross mortality was the improvement in hygiene and management on the rearing farm, and the mortality amongst the ducklings did not appear to be appreciably influenced by the persistence of carriers in the breeding flock.

It was determined if possible to find out to what extent egg transmission of the disease occurred, and 113 birds, which had given a positive reaction to the agglutination test, were brought to the laboratory for further experimental work. This work is still in progress, but the following is a brief summary of the results to date.

Seventy-nine of these carrier ducks have so far been examined, 74 of which had given a positive reaction to the agglutination test for *S. enteritidis gærtner,* and from these the corresponding organism

was recovered from the tissues of 39 ducks. The remaining five ducks had been shown on the initial test to be carriers of *S. typhi-murium* infection, and this organism was recovered from each of the five birds.

In most instances the organism was found to be confined to the ovary, this being the sole organ affected in 30 of the ducks examined. *S. enteritidis gærtner* was recovered from the ovary of 34 of the ducks examined, while *S. typhi-murium* was isolated from the ovary in three instances. Other sites from which the organisms were isolated were: old, encapsulated concretions of egg material in the peritoneal cavity, the gall-bladder wall, liver, spleen, heart wall, oviduct, and bowel. It is of interest to note that six ducks, from which *S. enteritidis gærtner* was recovered and one duck from which *S. typhi-murium* was isolated, had only reacted in the lowest dilution of 1 : 20 at the initial routine test.

Over 1,000 eggs laid by these carrier ducks were examined bacteriologically, but in no instance was either of the organisms under study recovered. This supports the findings of Hole, but is not in agreement with the results obtained by Scott, Warrack and Dalling, and Beller and Reinhardt.

A small breeding experiment was commenced late in the season, and two breeding pens of 12 ducks and a drake each, one containing carriers of *S. enteritidis gærtner* infection and the other carriers of *S. typhi-murium* infection, were set up. From the *S. typhi-murium* pen, 186 eggs were set, but fertility and hatchability were low, 93 eggs being infertile and 58 dead in shell. Only 35 ducklings hatched. From the *S. enteritidis gærtner* pen 70 eggs were set, 28 were infertile, 22 embryos died in shell and only 20 ducklings hatched. All the hatched ducklings were healthy and reared well without losses to six months old, and when blood-tested at the age of four months no reactors to the agglutination tests were found. All unhatched eggs were subjected to bacteriological examinations, but the organisms under study were not recovered in any instance.

Summary and Conclusion.

An extensive outbreak of Salmonellosis in ducklings is described in which the organisms responsible were found to be *S. typhi-murium* and *S. enteritidis gærtner*. The history of the outbreak, and the symptoms observed closely resemble those recorded by previous writers on this subject, and termed by them "Keel" disease.

Ducklings up to four weeks of age were most susceptible to natural infection. Few losses were experienced after this age, or in adult ducks, but a high percentage of carriers of both organisms was demonstrated in the breeding flock by means of the agglutination test.

Control measures outlined are described in detail, and with improvement of hygiene and general management on the rearing farm, the mortality was successfully reduced from 30 per cent. to below 3 per cent. Attempts to eradicate carriers in the breeding flock by blood-testing were unsuccessful, owing to lack of correlated effort on the part of the owners.

Breeding experiments carried out at the laboratory, and the cultural examination of eggs laid by carrier birds, gave negative results. This, together with observations on the course of the disease in the field tends to indicate that egg transmission was not the most important factor in the dissemination of infection, and that adult carriers served only as a possible means of introducing the disease occasionally, in spite of the fact that on *post-mortem* examination of 68 carrier birds the organisms were successfully isolated from the ovary on 37 occasions. Possibly, as with Pullorum disease, such carrier birds lay comparatively few eggs, with a low percentage of infected ones, or none at all.

In this, as in other outbreaks of Salmonella infection observed, vermin seem to play an important part in appearing to act as natural reservoirs and disseminators of infection.

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OBSERVATIONS ON COPPER POISONING

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Already there is a wide use of copper salts in agricultural and veterinary practice and the possibility of prophylactic use of copper for pregnant ewes in "swayback" areas, based on the recent association of copper deficiency with enzoötic ataxia in Australia (Bennetts and Chapman, 1937) and with "swayback" in lambs in this country (Dunlop and Wells, 1938), makes observations of acute and chronic copper poisoning in animals of some importance. The toxic nature of copper salts has been known for centuries, but the effect of small doses over a long period is not so well recognised; whereas some authorities have attempted to deny the existence of chronic copper poisoning altogether, others have attributed certain diseases specifically to this cause. As an instance, the observations of Mallory *et al.*, from 1925 onwards suggested a close relationship between hæmochromatosis in man and a possible chronic copper intoxication, although this view has found little support amongst other workers. The question as to whether small doses of copper ingested over a long period can exert toxic effects has been too often complicated by arguments from the special to the general case, and from an examination of the views expressed in the literature and experimental evidence at this laboratory, it seems that questions of magnitude and form of dosing, and species differences, at the very least must be carefully considered.

Acute Copper Poisoning.

Acute copper poisoning can be induced in all animals, since copper in large doses is a powerful protoplasmic poison, although the M.L.D. on a live weight basis may vary considerably in different species. In mammals, large doses of copper salts, administered *per os*, give rise to symptoms of nausea, vomiting, salivation, purgation, violent abdominal pains, convulsions, paralysis and collapse, ending in death. Cases of acute copper poisoning are rare but have been recorded when animals have been mistakenly given too large a therapeutic dose or obtain access to foods contaminated with copper salts, as may occur in the vineyards of Southern France after the use of copper insecticides. Reimers (1908) mentioned an occurrence of acute copper poisoning in foals, one of which died, after access to wheat treated with copper sulphate. Theiler (1912) in determining toxic doses of copper sulphate found 3 gm. or more in solution to be generally fatal to sheep of 80 lb. live weight. *Post-mortem* examinations showed marked gastroenteritis with congestion of spleen, liver and kidneys, and invariably the blood was coagulated at the time of death. The latter fact may explain the difficulty of obtaining blood from rabbits in the terminal phases of acute copper

poisoning (Eden and Green, 1939). The toxic dose of Theiler corresponds to about 20 mg. copper per kg. live weight. For rabbits, about 100 mg. copper as the sulphate was toxic when given by stomach tube in the present experiments, corresponding to 50 mg. per kg. live weight. The sheep thus seems to be more sensitive to oral administration of soluble copper salts. It is also more sensitive, on a live weight basis, when copper is introduced intravenously (Eden and Green, 1939).

The present paper offers further observations on copper poisoning including data on tissue analysis. In Table I are recorded distributions of the element over the soft tissues of the rabbit and sheep under normal conditions and under conditions of acute poisoning. All analyses are expressed on the fresh weight, samples of tissues being weighed out immediately after death and analysed for copper by a rapid modification of the diethyldithiocarbamate method.

Under normal conditions the copper content of the tissues is of much about the same order, being generally highest in the liver and lowest in the muscles. The figures are in fair agreement with those of Cunningham (1931) when recalculated on the same basis of fresh or of dry weight, except that he found the normal liver content much higher for the sheep. The relatively high liver copper content for the newly born, originally observed by McHargue (1925) is confirmed for the rabbit, but the high value rapidly sinks to normal within the first fortnight of life. Since the mother's milk presumably containing only the usual traces of copper characteristic of mammalian milk (0·1 p.p.m.), is the sole food over this period, during which the young rabbit may more than double its weight, this rapid fall suggests utilisation of stored reserves. The copper contents of the other organs examined do not differ greatly from those of the adult rabbit.

Under conditions of acute copper poisoning in the rabbit, the copper contents of all organs except muscle were increased, especially the lungs, liver and kidneys. However, the actual real increase in these organs is very small except for the liver, and since the circulating blood at death contained 3·2 mg. per 100 ml., the increase does not point to any marked absorption by any organ in the two hours between dosing and death. In the case of the two sheep poisoned by intravenous injection, the blood copper of one after four days was less than twice the normal value, and of the other, which died after five hours, was less than four times the normal. The analytical figures are thus not much affected by the presence of blood in the organs and the increased copper content is found to be principally in the liver, and to a lesser extent, the kidney, the remaining organs being practically normal. The same marked increase in liver and kidney copper contents was found in cases of chronic copper poisoning.

TABLE I.

COPPER CONTENT OF TISSUES.

Copper in mg. per 100 grammes fresh weight.

Condition.	SHEEP.			RABBITS.												
	Normal Adult.	*Died 5 Hours after i/v Injection of 100 mg. Cu.*	*Died 4 Days after i/v Injection of 80 mg. Cu.*	*Normal Adult.*	*Normal Adult.*	*Normal Adult.*	*Died 2 Hours after 200 mg. Cu by Stomach Tube.*	*Newly Born.*	*Newly Born.*	*1 Week Old.*	*2 Weeks Old.*	*2 Weeks Old.*	*3 Weeks Old.*	*4 Weeks Old.*	*5 Weeks Old.*	*8 Weeks Old.*
				1.	2.	3.		1.	2.	1.	2.					
Liver	1·22	12·7	17·6	0·42	0·50	0·78	3·0	2·8	1·5	1·9	0·87	0·36	0·27	0·54	0·37	0·23
Kidney	0·48	3·0	1·3	0·39	0·38	0·39	4·6	0·24				0·22	0·20	0·26	0·37	0·34
Muscle	0·21	0·25	0·20	0·14	0·17	0·08	0·22	0·22		0·20		0·25	0·16	0·16	0·17	0·11
Spleen	0·50	0·61	0·45	0·29			1·73									
Lung	0·48	0·60	0·52	0·24	0·39	0·25	2·71	0·27					0·17			
Heart...	0·50	0·48	0·59	0·39	0·40	0·31	1·15									
Bile				0·36			1·16									
Pancreas	0·25	0·22		0·33			1·23									

From the figures in the literature, the liver is the organ showing greatest variation in copper content under normal conditions. Cunningham (1931), in his extensive studies on twelve different species of animals, found that although there were variations from species to species, the livers showed the greatest degree of variation. and he reported figures of from 9 to 470 mg. per kg. dry matter. Allowing a 67 per cent. moisture content some of his figures expressed as mg. copper per 100 gm. fresh weight of liver would be, human 0·83; bovine (adult) 2·6; sheep (adult) 7·9; and rabbit 0·31. He drew particular attention to the high copper content of the nine samples of sheep's liver analysed but made no mention of the previous history of the sheep in regard to copper feeding or dosing, this being presumably unknown. Human liver, according to Hahn and Fairman (1936), on the basis of 142 analyses, has an average copper content of 0·61 mg. per 100 gm. fresh weight. Tompsett (1934) gave values for human liver ranging from 0·31 to 0·91 mg. per cent., the other organs examined showing copper contents of the same order as reported in Table I for the rabbit and sheep.

The Government Analyst for Queensland, in his 1936 report (*Analyst,* 1937), gave the normal copper content of sheep's liver as from 0·4 to 4·2 mg. per cent. fresh tissue, compared with 12·5 to 26·3 mg. per cent. for those sheep suffering from "black liver." Boughton and Hardy (1934) gave 0·48 to 4·19 mg. per cent. as the normal for four sheep. Dunlop Young (1937) recorded a series of livers from imported lambs, analysed in an attempt to decide whether copper present above certain concentrations was sufficient to warrant condemnation as unfit for human consumption. The analytical figures, recalculated from the dry matter basis on which they were expressed, range from 0·5 to 17 mg. per cent. fresh material, average 4 to 5. This average figure is substantially in agreement with that found by Cunningham although the upper values reported by Dunlop Young have not received confirmation elsewhere. Assuming that none of these data has been influenced by analytical or contamination errors, the copper content of sheep's liver shows the very wide range of 0·4 to 17 mg. per cent. fresh tissue. The figures given in Table I for the two cases of acute poisoning in sheep fall within this range and are thus not diagnostic of the known cause of death.

Blood copper data from sheep in an experiment on "Northumbrian Pine" have previously been reported (Eden, 1939), and an opportunity was given for an analysis of the livers of some of the sheep killed or dying in the course of that work. The data are given in Table II.

In control group D the liver copper values range from 0·22 to 9·3 mg. per cent. and in control group C the range is from 0·30 to 4·45, though in both these groups about 60 per cent. of the values lie below 1 mg. per cent. In group B the range is from 0·29 to 6·09 mg. per cent., again with 60 per cent. below 1 mg. per cent.,

TABLE II.
COPPER CONTENT OF SHEEP'S LIVER.
Copper in mg. per 100 grammes fresh weight.

Group.	*No.*	*Copper.*	*Group.*	*No.*	*Copper.*	*Group.*	*No.*	*Copper.*
D	98	0·88	C	77	0·30	A	293	14·3
D	108	0·27	C	85	0·80	A	299	16·1
D	112	0·99	C	88	0·57	A	503	1·44
D	115	0·86	C	91	0·61	B	36	0·29
D	126	3·98	C	181	0·41	B	49	0·72
D	240	0·84	C	199	0·77	B	58	1·04
D	241	0·28	C	225	0·53	B	61	1·51
D	245	0·59	C	327	2·91	B	213	1·72
D	251	3·13	C	332	4·45	B	307	0·59
D	252	0·38	C	336	0·87	B	316	0·83
D	254	2·15	C	337	1·36	B	320	6·09
D	257	0·38	C	338	0·83	B	321	0·58
D	261	1·04	C	339	1·65	B	516	0·57
D	263	1·92	C	341	1·06	E	133	10·40
D	547	0·22	C	345	0·34	E	154	4·52
D	549	3·34	C	535	0·38	E	233	5·39
D	551	0·61	C	538	0·72	E	238	3·32
D	552	0·68	C	544	0·45	E	264	28·50
D	555	0·22	C	610	1·37	E	270	4·15
D	603	3·84	C	769	1·29	E	274	9·95
D	604	9·30	A	25	6·35	E	275	6·90
D	724	7·95	A	204	1·18	E	280	6·96

C and D : Control Groups on natural grazing only ; B : Supplementary feeding (mineralised cake containing 52 p.p.m. copper) ; E. : Anthelmintic treatment (copper sulphate and nicotine) ; A : Supplementary feeding and anthelmintic treatment.

despite the doubling of the copper intake of the grazing by the feeding of half a pound of mineralised cake daily. Groups A and E which received relatively massive doses of copper sulphate in solution about once a month tend to show higher figures, although in group A two of the five values are below 1·5 mg. per cent. There were no gross pathological lesions in these livers correlated with the higher copper content, and although it is difficult to define what may or may not be regarded as "normal," there is no reason to consider the lowest figure of 0·22 and the highest of 28·6 mg. per cent. as being "abnormal" in any clinical or pathological sense of the term.

Figures such as 28·6, it is suggested, may be merely indicative of a high copper intake at some period of the animal's history. This width of range of liver copper values (0·22 to 28·6) is similar to the wide range of blood values previously reported in the experimental sheep (0·016 to 0·164 mg. per cent.) and the conclusion reached in the latter case that the large overall range had no particular significance may also apply to liver copper values. One point from these data is, however, clear; liver analysis *per se* can be of very little assistance in the diagnosis of copper poisoning in individual cases. In the two examples of fatal copper poisoning in sheep, reported in Table I, the liver figures were 12·7 and 17·6 mg. per cent., both lying

within what must be regarded as a permissible range for healthy sheep, although considerably higher than the "average normal." Diagnosis of acute copper poisoning would, therefore, seem to depend upon a combination of clinical symptoms, circumstantial evidence and *post-mortem* findings with confirmatory analytical evidence. High blood and urine figures would provide the most reliable index and since, as will be reported later, high urinary values only prevail during periods of high blood values, the contents of the bladder at death should receive special attention.

Chronic Copper Poisoning.

As already mentioned, the question of whether small quantities of copper ingested over a considerable period of time can exert toxic effects is a vexed one, and search of the literature shows there is a considerable diversity of opinion. Lander (1926) makes brief mention of chronic copper poisoning, stated to occur in the neighbourhood of copper smelting works amongst animals which show emaciation, weakness and general loss of condition, but adds that it is doubtful whether the disorder is not due to arsenic. Liver analysis of the animals supposed to have died from this cause failed to reveal a larger proportion of copper than that normally found. Cushny (1932) suggests that the chronic catarrh, colic and diarrhœa found amongst brass workers may not be due to copper but to lead, arsenic, and other poisons commonly associated with it. Wynter Blyth (1920) quotes Tschirch (1886) as saying, ". . . it appears the contention that there is no chronic copper poisoning in men or animals is at present uncontradicted; it is further to be considered proved that the small amounts of copper naturally in food or carefully introduced into food are not injurious to the health of those that take such food because the liver, kidneys and other organs excrete the copper through the urine and bile, and prevent a pernicious accumulation." Wynter Blyth supported the opinion that the question was not definitely settled. Elvehjem (1935) in a short reference to the toxicology of copper passes no judgment as to the occurrence of chronic copper poisoning, but states that after any organism has received sufficient for the needs of its metabolism, any additional copper is of no value and may be of some harm.

These opinions are representative of the position generally but also to be found in the literature are specific experiments leading to specific conclusions. Coulson, Remington and Lynch (1931) stated that the toxicity of small amounts of copper continuously ingested had been greatly overrated. They fed rats for a period of 12 months on a diet containing 52·7 mg. copper per 100 gm. and obtained no evidence of toxicity. This copper was either derived from "green" oysters or from copper sulphate artificially mixed with the food. They further stated that certain diets high in copper were not conducive to storage of the metal in the livers of experimental animals, and suggested that other investigators who claimed to have induced

"chronic copper poisoning" did so by using higher concentrations of copper, so passing from chronic to sub-acute intoxication. The distinction is of course arbitrary and related to time taken to develop symptoms. In the present paper chronic poisoning is regarded as the cumulative effect of successive non-toxic doses extending over at least a month.

Ellenberger and Hofmeister (1883) gave daily to sheep doses of copper sulphate varying from 0·5 to 3 grammes and found death to occur within periods of from 50 to 114 days. Baum and Seeliger (1898) repeated this work employing various copper salts and other species of animals. In isolated instances no clinical symptoms became evident but in most cases death occurred, preceded by emaciation, anorexia and weakness, with occasional instances of thirst, cramp and loss of hair. The general *post-mortem* findings were catarrhal inflammation and thickening of the stomach and small intestine and enlargement, fatty degeneration and necrosis of the liver and kidneys. The intensity of the changes was found to depend on the kind of copper preparation employed and on the species of animal.

Beijers (1932) reported chronic copper poisoning in sheep grazing in an orchard that had been sprayed with Bordeaux mixture. After several months clinical symptoms of marked anæmia and jaundice appeared, accompanied by blood in the fæces and urine. Degenerative changes were found in the kidneys and liver, the latter containing considerable quantities of copper.

A condition among sheep, characterised by generalised icterus and hæmoglobinuria, appeared in Texas, not transmissible by the usual agencies. Losses were moderately heavy. The sheep, grazing on range, had free access at their watering places to a mixture containing salt, tobacco dust and copper sulphate for the prevention and control of stomach worm infestation. Boughton and Hardy (1934) concluded that they were really dealing with chronic copper poisoning. They experimentally induced the disease by feeding normal sheep with the mineral mixture concerned, which contained 9 to 10 per cent. copper sulphate. The general clinical findings were loss of appetite, dullness, a yellowish skin, with the wool harsh and of dry appearance, hurried and shallow respirations, paleness of the mucous membrane, hæmoglobinuria and mild constipation. *Post-mortem* appearance showed a poor condition, a generalised icterus, chocolate colour and delayed coagulation of blood, with the serum tinted a light red or brown colour, containing hæmoglobin. The liver was enlarged, very friable and of yellowish colour, the kidneys enlarged with a black marbled surface on removal of the capsule, the spleen enlarged and of the colour and consistency of blackberry jam. Analyses of the livers of field cases and of those experimentally induced, showed values ranging from 14·7 and 81·2 mg. Cu per 100 gm. fresh tissue, compared with normal values of 0·48 to 4·19 mg. per cent. Blood smears showed marked anisocytosis, polychromasia

and punctate basophilia; in the later stages of the disease many nucleated red blood cells and up to 10 per cent. normoblasts were found, indicative of active blood regeneration. The stomachs of these animals were remarkable free from worms. Daily drenching of sheep with 100 c.c. of 1 per cent. copper sulphate solution did not produce death in under 30 days, showing that the danger of copper poisoning from routine drenching for control of stomach worms is very remote.

A similar condition in sheep, characterised by icterus and hæmaturia has long been known in the southern regions of France. The disease has been variously attributed to piroplasms and anoplasms, but Lafenêtre *et al.* (1935), could not confirm this. From the ætiology of the disease they made the suggestion that the condition is really a form of chronic copper poisoning, the source of copper being the sprays used as insecticides and fungicides on the vines amongst which the sheep were grazing. The chemical evidence was very meagre and the organs of only one ewe suffering from the condition were analysed. The liver contained 18·6 mg. copper oxide per 100 gm. fresh material, equivalent to about 14·9 mg. copper, and figures for the kidney and heart revealed values only about twice those normally found. The conclusions of Lafenêtre are very guarded, but it is stated that the condition seems to result from copper intoxication, since symptoms of experimental copper poisoning are very similar, and the "hæmorrhagic icterus" appeared simultaneously with heavy spraying of the vines. The possibilities of piroplasmosis, however, were not excluded.

The experiments of Boughton and Hardy in Texas are the most complete to date on chronic copper poisoning in sheep but were conducted mainly from the clinical aspect. A few analyses were made of the copper contents of livers of sheep dying naturally and under experiment, but beyond occasional hæmatological investigations no systematic biochemical studies were made. The present investigations were made from the aspect of blood and tissue chemistry while the clinical and pathological sides were less closely studied.

The possibility of chronic copper poisoning amongst sheep in this country under normal circumstances is rather remote, but the widespread use of copper sulphate makes it desirable that methods for its recognition should be available. A reported instance occurred six years ago when Bisset (1934) investigated periodic losses amongst sheep grazing within a mile of a smelting works in Wales. Symptoms included depression, dullness, emaciation, weakness and hæmaturia, and *post-mortem* examination revealed a brown or yellow liver, considerably enlarged; inflamed and congested kidneys and a gastro-intestinal catarrh. Chemical analyses of livers revealed in one case 59 mg. copper per 100 gm. fresh material, and in another 25 mg. per cent. Bisset stated that both copper and nickel were found in the herbage, but gave no figures proving an abnormal copper intake. No

arsenic was found but the possibility of arsenical poisoning was not completely excluded. There is no clear proof that death was due to copper poisoning, but the analyses quoted show high copper contents of the livers. It was stated that the sheep had never been dosed with any copper preparation so that the high liver copper figures could not have derived from this source.

Experimental Observations.

Several rabbits were put on to a diet containing 200 mg. copper per head per day as the sulphate, damped on to the food in solution and then dried at about 100° C. Three rabbits refused to eat the food, but a fourth, No. 3986, after refusing at first to eat, gradually became accustomed to the diet and after a few days was eating 70 gm. daily of a bran-oat mixture, containing the 200 mg. copper dried to the food. The food was thoroughly mixed before being offered to the animal. After 48 portions of food, corresponding to a total intake of 9·6 gm. copper above that normally present in the food, the rabbit was killed. All the organs appeared quite healthy on *post-mortem* examination. Thus, although 200 mg. copper given in a single dose by stomach tube was fatal within an hour or so, yet dried on to the food, the same quantity could be fed regularly without occasioning any ill-effects. In the same way, 100 mg. copper, an M.L.D. for a rabbit of 2 kg. live weight when given in solution by stomach tube, was dried on to the food and similar daily portions were fed for 295 days, after which the rabbit, No. 3987, died. The liver appeared normal but the spleen was grossly enlarged and pseudo-tuberculosis organisms were isolated on culture, so that death could not be attributed to the prolonged copper feeding. The results of tissue analysis of rabbits 3986 and 3987 are reported in Table III.

Table III.
Analysis of the Tissues of Rabbits after Continuous Copper Feeding.

Condition.	3986.		3987.	
	200 *mg. Copper Fed Dried on to Food for* 48 *Days.*		100 *mg. Copper Fed Dried on to Food for* 295 *Days.*	
	Copper in mg. per Whole Organ.	*Copper in mg. per* 100 *grammes.*	*Copper in mg. per Whole Organ.*	*Copper in mg. per* 100 *grammes.*
Liver	19·3	40·9	4·4	9·7
Kidney... ...	0·054	0·48	0·042	0·36
Muscle	—	0·15	—	0·12
Spleen	0·003	0·44	—	—
Lung	0·030	0·44	0·020	0·22
Heart	0·020	0·44	0·022	0·50
Bile	0·016	3·44	0·006	0·73
Pancreas ...	0·003	0·36	—	—
Blood	—	0·088*	—	—

*per 100 ml.

Despite the vast intake of copper the amount actually stored in the tissues is very small. In both cases the liver was the only organ reflecting in a marked degree the copper intake. Hall and McKay (1931) found that on prolonged feeding of copper acetate to rabbits, liver figures of the order of 75 to 100 mg. per cent. fresh weight were necessary before there was any evidence of pigment cirrhosis. The remaining tissues, except the bile of No. 3986, contained practically the normal amount of copper. It is interesting to note that the blood of this rabbit, even with such a high liver copper figure as 41 mg. per cent. was normal, showing that the influence of copper feeding upon blood copper level is comparatively slight. This point has already been shown with sheep under large scale conditions (Eden, 1939) and will be further exemplified in the experiments describing chronic copper intoxication in sheep.

In these experiments, the copper sulphate solution was merely dripped on to the food and not dried off as for rabbits or else was administered as a daily drench. More detailed studies were made in these cases. The sheep were weighed and bled each week, more often when necessary. Analyses were made of blood copper, sugar, inorganic phosphorus and hæmoglobin and of serum calcium and magnesium. The sheep were fed or dosed individually as far as possible and 1·5 gm. $CuSo_4$ $5H_2O$ (about 375 mg. copper, 7 or 8 mg. per kg. live weight) were given each day. Boughton and Hardy (1934) had shown that symptoms of poisoning could be produced within two or three months at this level. This quantity was greater than the usual therapeutic dose of 1 gm. (100 c.c. of 1 per cent. solution) but since it was desired to study various blood changes occurring during chronic poisoning rather than the minimum quantity of copper necessary to produce effects, the dose was suitable. Those sheep to which copper was fed damped on to concentrates took a few hours to consume their ration so that the concentration of copper in the tract would be less than by drenching. At times it was necessary to restrict the straw bedding, especially when the animals showed a disinclination to eat the copperised food. Water was always freely available. Continual adjustments had to be made in the quantity of food offered each day especially when the sheep were taking badly to the ration. Others, however, soon became used to the copper-containing food and there was little trouble with feeding until a day or so before death.

Sheep 845.

Sheep 845, a year-old wether, was put on to the experimental diet containing 1·5 gm. $CuSO_4$. $5H_2O$ per day on January 20th. This diet was refused for a day or two and normal food was given. From January 25th when the copperised food was again offered until February 3rd, only a small portion of the ration was consumed and from the 7th to 9th no food at all was eaten. On the 10th it was decided to drench daily with 1·5 gm. copper sulphate dissolved in 50 ml. water. The animal quickly took to normal food again and

ate quite well. It appeared normal for some weeks except for a pronounced tendency to drink more than usual and also to spend an unsual time in the recumbent position, possibly associated with the fact that it had previously suffered from a form of foot-rot. Dosing continued without incident until March 24th and was suspended from the 25th to the 29th, recommencing on the 30th. The animal now appeared to be very dull, ate very little and preferred the recumbent position. On April 5th the sheep was very unsteady on its feet after bleeding; the whole blood was a chocolate colour and the plasma indicated distinct hæmolysis. Bleedings were made periodically throughout the day and the final sample was taken on the 6th, when the animal could only rise with great difficulty and was obviously dying; it was found dead next morning. *Post-mortem* examination revealed a carcase in good condition, but with the skin and visceral fat markedly yellow. The liver was yellow and cirrhotic; the lungs were somewhat congested; there was slight inflammation in the abomasum and duodenum but the kidneys and remaining viscera appeared normal. In all, the sheep received nearly 20 gm. copper as the sulphate in solution over about seven weeks; symptoms and death are attributed to chronic copper poisoning. Weight records and blood analyses are given in Table IV.

TABLE IV.

SHEEP 845. WEIGHT RECORD AND BLOOD ANALYSES.

Date.	Weight.	Blood Values.				Serum Values.	
		Copper mg. per cent.	Sugar mg. per cent.	Inorganic P mg. per cent.	Haemoglobin grammes per cent.	Calcium mg. per cent.	Magnesium mg. per cent.
	lbs.						
Jan. 18th	125	0·065	56	3·9	12·0	9·9	2·5
Feb. 1st	118	0·131	64	4·5	9·7	9·9	2·6
„ *8th	112	0·109	45	6·2	10·0	9·8	2·4
„ 15th	119	0·105	63	6·0	8·2	10·1	3·0
„ 22nd	120	0·146	54	7·8	11·7	9·2	2·3
March 2nd	125	0·105	51	5·1	9·1	10·1	2·7
„ 8th	126	0·149	52	5·1	7·7	10·3	2·5
„ 15th	134	0·150	55	5·3	9·2	10·0	2·5
„ 22nd	131	0·141	59	7·6	9·9	10·0	2·7
„ 30th	133	0·123	49	6·2	10·7	10·7	2·4
April 5th	118	0·970	60	6·2	9·3	9·1	2·2
1 hour after dosing		0·97	—	—	—	—	—
2 hours after dosing		1·01	—	—	—	—	—
2½ „ „		1·00	—	—	—	—	—
4 „ „		0·99	—	—	—	—	—
5 „ „		0·90	—	—	—	—	—
6½ „ „		0·94	—	—	—	—	—
24 „ „		0·68	—	—	6·4	—	—

*Daily drenchings commenced on February 10th.

As is seen from Table IV, the calcium, magnesium and sugar values showed fluctuations within the normal range. Inorganic phosphorus values varied from 3·9 to 7·8 and hæmoglobin figures

showed unexplained arbitrary fluctuations, being lowest on the day of death. In the period during which the copper was incorporated on the food, the sheep lost weight rapidly, owing to its refusal to eat, but with normal food consumption during the drenching period, it put on weight until a week before death, when it again consumed less and lost about 15 lb. in weight. The blood copper values rose from the initial level of 0·065 to 0·131 mg. per cent. in the first fortnight of the experiment, but even after drenching was commenced the values only fluctuated from 0·105 to 0·150 mg. per cent. over seven weeks, showing that blood copper is relatively little affected by non-toxic quantities of copper even when administered in a form most suitable for absorption. In the week before death the figure rose to seven or eight times the previous values and so persisted until death. The periodic bleedings on April 5th were made to observe the behaviour at shorter intervals after dosing. Within the limits of experimental error no significant deviation from the high "premortal level" was observed during the hours after drenching. Next morning the value seemed to be falling again, but no sample was obtained immediately before death. Results of tissue analysis are reported in Table V.

TABLE V.

SHEEP 845. ANALYSIS OF THE TISSUES.

	Copper in Whole Organ.	*Copper per 100 grammes.*
	mg.	mg.
Liver	519·3	61·8
Kidneys	55·5	27·3
Muscle	—	0·15
Spleen	3·28	2·52
Lungs	—	0·84
Heart	1·63	0·51
Pancreas	—	0·69
Brain	—	0·23
Bile	—	0·43 mg./100 ml.

The high copper content of liver and kidneys is noteworthy, there being over half a gramme of copper as such stored in the liver as a whole. The kidney figure is about 60 times the usual normal value. The remaining organs are about normal, except for the spleen where destruction of blood (of high copper content) was probably occurring. The bile figure is also high, perhaps reflecting that of the liver. It is interesting to note that muscular tissue, greatest in mass, appears to store practically no copper.

Sheep 750.

This wether of the same age as No. 845 was put on to a high copper diet on January 18th. Although at first it showed disinclination to eat, normal food consumption was resumed within a week and 1·5 gm. $CuSO_4$. $5H_2O$ damped on the ration were ingested continuously, except for a break from March 25th to 29th. On

April 11th, some of the ration was uneaten and on the 12th the sheep was very dull, showing a strong disinclination to move, with arching of the back and lowering of the head when made to stand. The blood was chocolate in colour and the serum indicated marked hæmolysis. On April 13th, the animal was obviously dying and there was profuse salivation from the mouth. No abnormal cells, such as described by Boughton and Hardy, were observed in a blood smear. On the 14th it was found dead and *post-mortem* examination showed a carcase in fair condition with the skin again showing signs of extensive yellow pigmentation. A large amount of abdominal fluid of reddish tinge was present. The alimentary tract was empty of food but apparently normal. The liver was markedly lobulated, the spleen greatly enlarged and the kidneys deeply congested. Heart and lungs were normal, with a fair amount of pericardial fat. In Table VI are recorded the weights, blood data and results of tissue analysis.

TABLE VI.

SHEEP 750. RECORDS OF WEIGHT, BLOOD AND TISSUE ANALYSIS.

		Blood Values.				*Serum Values.*	
Date.	*Weight.*	*Copper*	*Sugar*	*Inorganic P*	*Haemoglobin*	*Calcium*	*Magnesium*
		mg.	*mg.*	*mg.*	*grammes*	*mg.*	*mg.*
	lbs.	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*
Jan. 18th	141	0·095	53	6·3	12·8	9·4	3·0
Feb. 1st	136	0·110	49	6·4	11·1	9·0	2·9
„ 8th	130	0·119	53	5·0	11·9	9·0	2·9
„ 15th	134	0·156	49	4·9	10·5	9·2	2·8
„ 22nd	129	0·150	48	7·4	13·2	9·1	2·4
March 2nd	129	0·149	49	5·2	9·9	9·4	2·9
„ 8th	129	0·150	46	6·0	10·4	9·5	2·7
„ 15th	135	0·165	56	7·2	12·3	9·6	2·7
„ 22nd	138	0·160	55	5·7	10·6	10·2	3·2
„ 30th	143	0·170	48	6·2	10·9	11·1	2·6
April 5th	139	0·185	55	5·7	11·2	10·7	2·9
„ 12th	136	1·28	42	5·6	8·5	9·7	5·2
5 p.m.		1·15	46	5·6	7·4	9·9	5·9

TISSUE ANALYSIS.

	Copper in Whole Organ.	*Copper per 100 grammes.*
	mg.	mg.
Liver	204·9	35·7
Kidneys	32·6	13·2
Muscle	—	0·21
Spleen	2·02	0·63
Lungs	—	1·97
Heart	—	0·67
Brain	—	0·41

The calcium values showed a tendency to rise slightly about three weeks before death although remaining within the normal range. Magnesium values remained fairly constant until the day of death when they rose to double the normal value; no explanation can at

present be offered for this rise. The sugar and inorganic phosphorus levels throughout were normal and hæmoglobin values showed the usual fluctuations within the normal range, with a noticeable drop within the last week of life. The live weight remained steady throughout. Blood copper values showed a slight progressive elevation with a rapid "premortal rise" to a high level at the time of death.

In the tissues, high copper values were again shown for liver and kidneys. Of the other organs the lungs showed the greatest of changes, but since these highly vascular organs were bathed with blood of a high "premortal" copper content at death, the change is relatively insignificant. The liver figure, it may be noticed, was not as high as with the previous sheep, although receiving copper for a longer period; possibly the difference may have been related to the difference in the forms in which the copper was taken into the animals, drenching and incorporated on to the food.

Sheep 836.

In contrast to the previous sheep, another wether, No. 836, provided an example of steadfast refusal to eat food damped with copper sulphate and finally had to be drenched in the same way as No. 845. The sheep was put on the same diet as others on February 10th, but after accepting the copperised ration at first, refused most of it by the 19th. Some hay was being eaten but very little of the copperised concentrates and by March 1st the latter had to be thrown out and a fresh supply given. Feeding habits were then erratic. The animal would eat for a day or two and then go off feed. After several abortive attempts to induce regular consumption, it was decided on April 20th to administer the copper daily as a drench food and to feed a normal ration. Blood-stained fæces were passed on April 21st. The sheep became very dull towards the end of the month and on May 3rd the serum showed noticeable hæmolysis. Very little food was now eaten, the sheep remaining dull and recumbent. No signs of hæmolysis were observed in the serum at the blood sampling on May 18th, but the animal was very weak and appeared to be sinking. It was found dead on the 23rd and on *post-mortem* examination the principal points of interest were congestion of the lungs and kidneys and a very yellow and friable liver. The intestines were practically empty except for a hard mass of fæces at the ileo-cæcal valve. In Table VII are recorded the usual data on live weight, blood and tissue analysis.

The progressive emaciation, as shown by the live weight figures, reflects the continued anorexia over the experimental period. The blood copper figures over the period of attempted copper feeding are relatively constant but after systematic drenching a slow rise to twice the normal value was manifested. Venous return was so sluggish on the day of death that it was impossible to get a blood sample, and as the previous sample had been taken five days before, the characteristic "premortal rise" was not detected although it

TABLE VII.

SHEEP 836. RECORD OF LIVE WEIGHT, BLOOD AND TISSUE ANALYSIS.

Date.	Live Weight.	Blood Values.				Serum Values.	
		Copper mg. per cent.	Sugar mg. per cent.	inorganic P mg. per cent.	Haemoglobin grammes per cent.	Calcium mg. per cent.	Magnesium mg. per cent.
	lbs.						
Feb. 9th	110	0·106	49	4·4	11·6	10·0	1·5
„ 15th	104	0·158	52	5·5	11·5	9·6	1·8
„ 22nd	103	0·136	42	6·5	12·8	8·4	1·1
March 2nd	94	0·141	42	5·5	9·2	8·2	1·3
„ 8th	96	0·151	59	4·0	8·3	10·2	2·1
„ 15th	97	0·154	52	5·1	11·4	10·3	2·5
„ 22nd	94	0·146	51	3·8	11·0	9·6	2·8
„ 30th	103	0·128	50	4·2	11·9	10·3	2·2
April 5th	96	0·156	39	5·3	12·8	9·2	2·2
„ 12th	86	0·157	41	5·6	12·7	9·6	2·1
„ 20th	89	0·150	66	3·2	14·6	9·2	2·7
„ 26th	82	0·154	51	3·5	9·8	10·1	2·0
May 3rd	78	0·238	62	4·5	8·0	9·2	2·1
„ 5th	—	0·184	—	—	6·1	—	—
„ 7th	—	0·192	—	—	6·7	—	—
„ 10th	64	0·270	58	5·6	7·8	10·7	2·2
„ 18th	62	0·229	57	4·9	8·5	10·8	2·3

TISSUE ANALYSIS.

	Copper per Whole Organ.	Copper per 100 grammes.
	mg.	mg.
Liver	673·2	134·9
Kidney	32·5	20·7
Muscle	—	0·21
Spleen	0·48	1·37
Lungs	38·7	4·91
Heart	1·2	0·47
Bile	—	2·50 mg./100 ml.

presumably occurred. The sugar values fluctuated as usual although somewhat lower during the period of attempted copper feeding than during the subsequent drenching. Inorganic phosphorus values call for no particular comment. There seems to be no obvious explanation for the variations in hæmoglobin figures over the first period although the low later values are worth noting. The fluctuation of serum calcium and particularly of magnesium was greater than previously encountered. Over the first month the magnesium figures were subnormal, down even to 1·1 mg. per cent., but thereafter the variations were within normal limits. No explanation is offered.

Of the tissue figures, the most striking is the enormous liver copper value, with the concomitant rise in biliary copper. The figure of 135 mg. per cent. was the highest encountered in the present series and may perhaps be due to the erratic food consumption. Partial absence of food from the alimentary tract during the 30 days'

drenching would allow more pronounced flushing of the duodenum with soluble copper and a higher degree of absorption. The lung figures were also above normal but since the blood copper value at death was not known, it is impossible to state how far they were influenced by "premortal rise" of the blood copper.

Sheep 872 *and* 877.

Two rams, discarded from an independent experiment and no longer required for breeding purposes were used for the concluding experiments in the present series. Separate accommodation was not at first available and the two rams were fed together with 3 gm. $CuSO_4$. $5H_2O$ in solution mixed on to a palatable concentrate mixture of flaked maize, oats, and linseed cake, with hay supplied *ad lib.*, after the copperised ration had been consumed. Feeding commenced on April 10th and the two rams readily took to this ration which was fed daily except for a break of three days when personal attention could not be given. On May 24th the two rams were separated and individual feeding adopted. On the 28th No. 877 left part of his ration and still more the following day. This loss of appetite was quite sudden and in other respects the ram was normal. On the 30th no food was eaten and the animal died between 9 and 10 a.m. *Post-mortem* examination revealed apparently normal spleen and kidneys but a very yellow, cirrhotic liver. No ascites was noticed. Petechiæ occurred on the myocardium and sternum. The colon was packed with hard blood-stained fæces without noticeable inflammation of the gut wall itself.

No. 872 in the adjacent pen went off feed on May 31st and no copper was ingested that day. More copperised food was given on June 1st but none on the 2nd. All the previous food was cleaned out on the 3rd and replaced with fresh. On the 5th the ram was dull and recumbent but continued to eat until the 10th when it became very listless, refusing food and dying in the afternoon of the 11th. *Post-mortem* examination revealed marked petechiæ and hæmorrhages on the myocardium and pleural linings. The liver was very congested with well marked lobulation and the kidneys were swollen and congested. The mucous membrane of the rumen was partially detached from the supporting tissue without much inflammation. In Table VIII are recorded the live weight blood and tissue copper data for both rams.

As Table VII shows, the animals lost no weight over the experimental period nor were there any striking changes in the blood sugar and inorganic phosphorus values. The magnesium figures showed an upward tendency at the time of death as was noticed in the serum of Sheep 750, but the calcium values remained relatively steady, with slight hypocalcæmia in No. 877. The changes in hæmoglobin values in No. 872 followed the usual fluctuations with a downward trend towards death in the case of No. 877. The slow rise of blood copper was again noticed in both sheep as the experiment progressed, and once again there was the "premortal rise" in blood copper. The

animals died in relatively shorter time than did the preceding animals the experiments lasting 34 and 51 days for Sheep 877 and 872 respectively with copper intakes of 15 and 19 gm.

TABLE VIII.

RAMS 872 AND 877. LIVE WEIGHT, BLOOD AND TISSUE DATA.

Date.	Live Weight. lbs.	Blood Values. Copper mg. per cent.	Sugar mg. per cent.	Inorganic P mg. per cent.	Haemoglobin grammes per cent.	Serum Values. Calcium mg. per cent.	Magnesium mg. per cent.
No. 872							
April 20th	111	0·095	59	6·5	12·9	9·6	2·6
„ 26th	108	0·096	56	6·4	10·3	9·3	2·9
May 3rd	103	0·190	60	4·2	12·6	9·5	2·3
„ 10th	101	0·160	44	3·4	12·8	10·3	2·3
„ 18th	105	0·197	55	5·9	10·1	10·2	3·2
„ 24th	107	0·217	59	4·7	8·0	9·9	2·5
„ 31st	108	0·238	55	3·2	12·4	9·1	2·1
June 7th	109	0·194	54	5·9	11·7	9·9	3·6
„ 10th	—	0·537	—	—	11·4	—	—
No. 877							
April 20th	73	0·078	57	7·6	10·4	8·6	3·1
„ 26th	67	0·106	53	6·6	10·2	8·8	2·9
May 3rd	74	0·095	57	6·0	11·1	8·7	3·1
„ 10th	73	0·106	42	6·4	10·4	8·5	3·3
„ 18th	74	0·160	49	5·4	9·2	9·0	3·3
„ 24th	80	0·171	49	5·6	7·8	8·0	3·8
„ 31st	—	1·60*	—	—	—	—	—

**Post-mortem* sample.

TISSUE ANALYSIS.

	No. 872. Copper per Whole Organ. mg.	No. 872. Copper per 100 grammes. mg.	No. 877. Copper per Whole Organ. mg.	No. 877. Copper per 100 grammes. mg.
Liver	312·7	26·0	226·5	29·4
Kidney	7·5	15·7	24·0	18·8
Muscle	—	0·14	—	0·52
Spleen	2·06	1·84	0·50	1·37
Lung	13·34	1·09	9·5	1·51
Heart	1·82	0·52	1·83	0·83
Bile	—	0·25*	—	0·09*
Pancreas	—	1·14	—	0·46
Mediastinal lymph glands... ...	—	0·67	—	—

*mg./100 ml.

The values for liver copper, although high, are not higher than for some of the healthy sheep reported in Table II, once again supporting the statement that high liver copper figures *per se* are of little diagnostic value beyond indicating an abnormal level of

copper intake. Death occurred in these two cases at a somewhat earlier stage and if copper storage is a gradual process there may not have been time for the liver to load up to the same degree. The kidney figures are high and kidney analysis would seem to furnish a better indication of chronic copper poisoning than the liver. The remaining tissues have figures of the same order as those found previously with one interesting exception, the muscle of No. 877, in which the copper figure of 0·52 mg. per cent. was over twice as high as any other muscle figure recorded in the present series, although if the copper be expressed in absolute terms the real quantity of copper stored cannot be very great; this may probably be due to the high blood copper figure at death (1·60 mg. per 100 ml.) although the muscle figure in other cases of high blood values have been relatively much lower.

One other example of death following continuous copper administration but by the venous route, is worthy of mention although unfortunately the course of events was not closely studied. In connection with another investigation involving blood magnesium, a ewe, No. 691, was daily injected with known quantities of copper (as the sulphate) made up with normal saline. After ten injections had been given the condition of the jugular veins was unsuitable for further injection and the experiment was discontinued. Shortly thereafter the ewe was dull and off-colour, eating badly and possessing great thirst. It died 17 days after cessation of copper injections but the behaviour escaped close personal attention since the animal was regarded as no longer under experiment. *Post-mortem,* however, revealed pronounced pneumonia and it is tentatively suggested that the lung lesions were secondary to delayed copper poisoning. If this suggestion is valid, the delay in appearance of symptoms of dullness, inappetence and thirst, is interesting as no further copper had been given for a considerable period before they appeared. On the other hand, both symptoms and death may perhaps be attributed entirely

TABLE IX.

EWE 691. SERUM COPPER FOLLOWING INTRAVENOUS INJECTION.

Date.	*Treatment.*	*Copper in mg./100 ml. Serum.*
April 23rd	None	0·078
„ 24th	„	0·093
„ 26th	„	0·086
„ 27th	Injection of 25 mg. copper	0·076
„ 28th	„ 50 „ „	0·078
„ 29th	„ 50 „ „	0·102
„ 30th	„ 50 „ „	0·086
May 1st	„ 50 „ „	0·089
„ 3rd	„ 50 „ „	0·090
„ 4th	„ 50 „ „	0·097
„ 5th	„ 50 „ „	0·149
„ 6th	„ 50 „ „	0·150
„ 7th	„ 50 „ „	0·169

to incidental pneumonia. The sample of liver taken at *post-mortem* contained 58·7 mg. copper per 100 gm. Further confirmation of the rapid return of blood copper to normal following injection (Eden and Green, 1939) is evidenced by the figures in Table IX, in which each value is that of a blood taken 24 hours after the preceding injection.

Discussion.

There appears to be considerable difficulty in inducing chronic poisoning in rabbits and sheep by addition of copper salts to food and relying on voluntary consumption. In the case of sheep the animals showed considerable aversion to food damped with dilute copper sulphate and given a free choice of the same food, with and without copper, the copper-free food was invariably selected. This suggests that under practical conditions sheep would avoid, as far as possible, food heavily contaminated by accidental means, as in the case of spraying with Bordeaux mixture. Some sheep, however, will eat copperised food when other food is withheld and in the case of the chronic copper poisoning recorded by Boughton and Hardy in Texas, the salt present in the copperised mixture left at the watering places for anthelmintic purposes may have been the inducement for consumption.

Chronic copper poisoning was experimentally induced in three sheep by damping a solution of 1·5 gm. of copper sulphate on to a daily ration of palatable concentrates, and in two sheep by daily drenching of the same quantity in 50 to 100 c.c. of water. Death resulted in from 30 to 80 days. Clinical symptoms were rather indefinite and only pronounced a few days before death, then consisting merely in dullness, anorexia and undue thirst, with icterus in the final stages. As copper administration proceeded there was a gradual rise in blood copper to about twice the initial level, until a day or two before death, when a sharp "premortal rise," to about ten times the initial level, occurred. This "premortal rise" is presumably due to a sudden breakdown in the mechanisms for elimination and storage of copper, associated with failure of liver and kidney function, since in the normal animal, blood copper, if raised to still higher levels by intravenous injection, rapidly returns to normal (Eden and Green, 1939).

No characteristic changes in blood sugar, inorganic phosphorus, calcium and magnesium occurred during development of chronic copper poisoning, although in some cases the serum magnesium rose shortly before death. Hæmoglobin values showed considerable fluctuations with a trend to lower values towards fatal termination. In some cases hæmolysis was noted in the serum.

The general features of *post-mortem* examination were a yellow, cirrhotic liver showing fatty degeneration or infiltration, swollen and congested kidneys and marked icterus. The liver lesions were associated with a high copper content, 25 to 135 mg. copper per 100 gm.

fresh tissue. The kidneys also showed marked increase in copper content, but elevation in other organs was slight and explicable on the high premortal rise of blood copper.

Without attempting to generalise too far, the main features in the course of chronic copper intoxication would appear to be (*a*) relatively limited absorption of copper from the intestinal tract even when the intake of copper is high; (*b*) adequate elimination of absorbed copper in bile and urine and possibly by intestinal wall, with limited storage, particularly in the liver, in such a way that blood copper does not rise materially in the early stages; (*c*) gradual functional damage, particularly to liver and kidneys, with slowly rising blood copper; (*d*) sudden breakdown of eliminative mechanisms, with pronounced lesions in liver and kidneys, associated with icterus and with sharp premortal rise of blood copper which itself may contribute to exitus.

A few experiments were also carried out on rabbits, in this case drying the copper sulphate on to the food at 100° C. after damping on as solution, instead of feeding direct in damped condition as in the case of sheep. This simple drying procedure appeared to reduce toxicity enormously. Although several rabbits refused to eat the copperised food, two animals rapidly overcame the initial aversion and consumed their daily ration completely for long periods without developing chronic copper poisoning, despite the incorporation of the relatively enormous amount of 0·4 gm. $CuSO_4 . 5H_2O$, equivalent to 100 mg. copper. This dose, if given in solution by stomach tube, in such a way that the duodenum is suddenly flushed with soluble copper, is frequently fatal within a few hours. Yet one rabbit was continued upon the same quantity of the same solution *dried* on to the food daily for 295 days and another for 48 days on *double* this quantity. In both cases there was storage of appreciable quantities of copper in the liver, the values per 100 gm. fresh material being 9 and 41 mg. respectively, but no signs of chronic copper poisoning developed, indicating that such absorption as did occur from the tract could be adequately dealt with by the eliminative mechanisms. Copper sulphate readily forms a loose combination with proteins of food and the presumption is that the simple procedure of *drying* rendered these insoluble. Liberation in available in the course of digestion would then be slow and it is presumed that this occurred in a portion of the tract at which formation of insoluble copper sulphide was possible, or that the peculiar refection habits of the rabbit (Eden, 1940) provided a method of detoxication by formation of inert copper sulphide from sulphuretted hydrogen in the refected fæces.

The observation, however, is of interest as showing that comparatively small changes in the form in which the copper occurs in food may make enormous differences in its availability and hence in its tendency to induce chronic intoxication. The experiment of *drying* the concentrates damped with copper sulphate solution, so

effective in reducing toxicity in the case of the rabbit, has not yet been repeated in the case of the sheep.

In this country, the possibilities of occurrence of chronic copper intoxication in agricultural practice are small, and since animals are not, as a rule, allowed to graze where copper is employed for spraying, the condition is rarely encountered. The increasing use of copper salts, however, and the associated possibilities of intoxication make it necessary that the condition shall be recognised when it occurs. Since acute intoxication can occur without rise in liver copper beyond the upper limit of so-called "normal" values, liver analysis *per se* is of uncertain significance. Elimination of copper in the urine, however, is high at periods of high blood copper and urine in the bladder at death probably offers the most useful analytical criterion.

Summary.

The copper content of the fresh tissues of normal adult rabbits and sheep lies between about 0·1 and 0·5 mg. per cent. except for sheep's liver which may have a range of from 0·2 to 29 mg. per cent. without any evidence of associated pathological change, although the greater proportion of values appear to lie under 2 mg. per 100 gm. Under conditions of acute copper poisoning the principal changes in the copper content of tissues lie in greatly increased values for liver and kidney.

The relatively high liver content of the newly born animal is confirmed for rabbits, the figure falling to the adult level long before weaning, suggesting the mobilisation of this copper for physiological purposes.

The relevant literature on chronic copper poisoning is reviewed. Attempts to induce this condition in rabbits by feeding copper sulphate *dried* on to food were unsuccessful although evidence of considerable copper storage in the liver was obtained. Chronic intoxication was induced in sheep by daily quantities of 1·5 gm. copper sulphate either in the form of a drench or damped on to a palatable mixture of concentrates, the condition developing in periods of from 30 to 80 days. Clinical symptoms included dullness, depression and anorexia and *post-mortem* examination showed generally icterus, cirrhotic liver and swollen and congested kidneys.

Chemical studies on the blood during the course of chronic intoxication showed a slow rise in blood copper content to about twice the initial value until a day or two before death when a sharp "premortal" rise to about ten times the normal value occurred. There were no significant changes in blood sugar, inorganic phosphorus and calcium values, and only a slight rise in serum magnesium content at the terminal phase. Hæmoglobin values showed considerable fluctuations. The copper content of the liver and, to a lesser extent, the kidneys were very considerably increased but relatively little change occurred in that of the remaining soft tissues examined.

Various factors such as form of administration of copper and species of animal are considered in the induction of experimental chronic copper poisoning and the course of the disease is generally discussed. The invalidity of diagnosis of copper intoxication, either acute or chronic, by liver analysis *per se* is emphasised.

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A NOTE ON PRELIMINARY OBSERVATIONS ON THE INFLUENCE OF DIET ON RESISTANCE TO TUBERCULOSIS IN PIGS.

By William M. McKay, Ph.D., M.R.C.V.S.

That diet exerts a very great influence on the incidence of tuberculosis has long been accepted as a factor of major importance in its control. A survey of the literature on the subject shows, however, that while much has been done in the way of dietetic treatment of cases, and the study of the effects of the various vitamins on the disease, very little experimental work has been undertaken to put the thesis to experimental proof.

It is known, however, that the malnutrition in Germany during the Great War resulted in a great increase in the death-rate from tuberculosis.

Baldwin (1925), discussing the ætiology of tuberculosis, stated that poverty was the most important single element in the development of tuberculosis. Consumption, he stated, was the social disease *par excellence* at the terminal stage of poverty, and quoted the figures of Korosi for Budapest, where among 10,000 living inhabitants, 40 well-to-do persons, 62·7 moderately well-to-do, 77·7 poor persons and 97 paupers died of consumption.

Overland (1935) reported that tuberculin tests of recruits in Norway in 1911 and 1934 showed little change in the number of positive and negative reactors, while the mortality from tuberculosis had diminished greatly. This pointed to the fact that although there was the same proportion of infections resistance to the disease had increased. The author considered this due to improved nutritional factors and not to housing. He supported this contention by statistical study of two groups of soldiers housed in different barracks. One group was housed in modern barracks over a period of years, and the other group in particularly bad conditions. The group in the good barracks had the highest sickness rate until 1927, when after improvement in the diet it became normal.

Schutze and Zilva (1927), using guinea-pigs inoculated subcutaneously with 0·1 mg. of living tubercle culture, found that those given an abundant diet lived twice as long as those on the restricted diet, and commented that the death-rate provided evidence of the deleterious effect of a restricted diet on the course of tuberculous disease.

Schmidt-Lange (1935) described a series of experiments on guinea-pigs, in which half were given a strictly limited diet, and the other half double the amount. Both groups were inoculated with human tubercle bacilli of low virulence. The results were classified according to *post-mortem* and histological findings and the extent of

infection so estimated was 63 per cent. higher in the underfed animals.

EXPERIMENTAL.

The following preliminary experiment was devised to show if pigs fed on a poor diet differed in their resistance to tubercle bacilli given by mouth from pigs fed on a good diet.

The experiment was carried out at the Rowett Research Institute during 1937.

Sixteen pigs, about ten weeks old, all negative to the double intradermal tuberculin test, were divided into two groups.

After a preliminary fortnight on normal rations, one group was put on a diet deficient in calcium and vitamins A and D, and the other group was put on a diet richer in calcium and vitamins A and D. The composition of the rations is given below.

DIETS USED.

Poor Diet.

	Percentage
Fine Sharps	46·0
Oatmeal	46·0
Ex. earth nut cake meal	7·5
Salt	0·5
Protein	17·0
Ca	0·37
P	0·56

Good Diet.

Fine Sharps	40·0
Ground barley	30·0
Flaked maize...	20·0
White fish meal	10·0
1 lb. separated milk per lb. meals	
¼ lb. green food per pig daily	
Protein	21·8
Ca	1·16
P	0·82

The diets were fed to appetite. The pigs were kept on the two diets till a marked difference in weight was observed between the two groups. During this period, three pigs in each group died of intercurrent disease: at *post-mortem* they were found not to have tuberculosis. Two pigs in the group on the good diet were also affected but recovered; thereafter they were permanently subnormal in weight.

After 50 days on the two diets a marked difference in weight in the two groups was observed. At this stage those on the good diet averaged 178 lb., those on the poor diet 87 lb. The pigs were now each given by mouth 1 mg. of pure T.B. culture in half a pint of milk and continued as before on the two diets. After 47 days

further, one of the abnormal pigs on the good diet died, and was found to have much T.B. at *post-mortem*. After 82 days, one of the pigs on the poor diet had to be killed and was found to have marked T.B. at *post-mortem*. After 87 days, the rest of the pigs were killed and examined. Only one pig in the group on the poor diet was in moderately good condition. The rest were dirty and unthrifty and had rickets. At *post-mortem* they all had general enlargement and intensive caseation of lymph glands with destruction of gland tissue and little attempt to limit the disease. The abnormal pig which survived in the group on the good diet was dirty and unthrifty, but the other three were entirely normal in appearance and in good condition. The abnormal animal showed fairly intensive involvement of lymph glands, but in the other three animals, there was much less evidence of infection. In the glands involved, the infection was limited to circumscribed areas and these animals would almost certainly have survived.

Although this experiment was intended to be a preliminary one and was purposely done on a small scale, it definitely suggests that resistance to T.B. can be enhanced by giving a good ration. Two of the pigs in the group on the good diet which came to *post-mortem* were definitely abnormal before the infection was administered. The rest of the animals in this group resisted the organism in a way which suggests that they would have probably overcome it, while in all the animals on the poor diet there was little or no check imposed on the infection.

Acknowledgments.

I have to thank Sir John B. Orr for much advice and for the hospitality of the Rowett Research Institute where the experiment was undertaken.

I am greatly indebted to Dr. James Irving, Head of the Physiology Department at the Rowett Institute, and to Dr. John Smith, Director of the City Hospital Laboratory, Aberdeen, for much assistance and advice.

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MULTIPLE MALIGNANT AND BENIGN NEOPLASMS IN A DOG WITH SECONDARY TUMOUR OBSTRUCTION OF THE PORTAL VEIN WITHOUT ASCITES.

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Multiple primary tumour formations are admittedly not infrequent in man (Willis, 1934) and domestic animals (Cohrs, 1927). Yet this instance of co-existent sarcoma, carcinoma and a benign neoplasm in a dog is considered worthy of description because of the absence of ascites in spite of marked obstruction of the portal vein by secondary sarcoma. In addition, the minute size of the other primary (carcinoma of the eyelid) emphasises the care necessary in anatomical diagnosis, as the metastasis from this tumour in cervical nodes, might easily have passed for an example of paradoxical metastasis of the sarcoma due to transpulmonary passage of tumour cells.

Subject.—Sealyham dog of 8 to 9 years with vague history of ill-health for a year; the animal was killed by HCN subsequent to a diagnosis of an inoperable abdominal tumour.

Anatomical Findings.

These can be conveniently given under four separate headings relating to the three tumour types and other findings.

(*a*) Large irregular primary sarcoma (9 × 8 × 5 cm.) in the sublumbar and subsacral retroperitoneal region intimately adherent to, but not invading, the vertebræ; ventral displacement of the pelvic organs and lateral displacement of the abdominal aorta and caudal vena cava; enclosure and compression of the left ureter with consequent hydronephrosis; cranial contiguity spread of the growth to the lesser omentum with invasion and obstruction of the portal vein; discrete umbilicated metastatic nodule (7·5 × 7 × 5 cm.) in the left lateral lobe of the liver; single metastatic nodule (5 × 4 × 3 cm.) in the left inguinal region discontinuous with the primary; no ascites, no splenomegaly and no œsophageal or rectal varices; no pulmonary metastases.

(*b*) Minute (0·4 × 0·2 cm.) tumour on the conjunctival surface near the margin of the right upper eyelid with metastases in the two right superior cervical lymph nodes.

(*c*) Incidental findings included œdema and congestion of the lungs; slight prostatic enlargement; a few flecks of fat necrosis in the pancreas; the brain appeared normal.

Microscopical Findings.

(*a*) *Retroperitoneal tumour.*—The primary growth consisted of sheets of cells closely packed in an indeterminate manner and supported by a fine argyrophil reticulum. The tumour was not highly vascular and, though there were numerous areas of necrosis, hæmorrhage was not conspicuous. The cells were mainly spindle-shaped, short fusiform or large and round in type, while bi- and tri-nucleated giant cells were not infrequent (Fig. 1); lymphocytes were scanty. Mitotic figures were exceedingly numerous.

The appearances were in many ways similar to those of highly cellular periosteal sarcomata, and in view of the intimate attachment to the vertebræ, the possibility of such an origin must be considered. There was no other indication of the exact site of origin and the alternative is to regard it as a mixed or polymorphic cell sarcoma arising from retroperitoneal connective tissue.

The structure of the single large liver metastasis was identical with that of the primary (Fig. 2). In addition, numerous branches of the portal vein in parts of the liver, remote from the secondary, were found to contain non-adherent tumour emboli (Fig. 3).

The obstructed portal vein was encircled and filled by tumour cells which in the original lumen were very loosely arranged and interspersed with masses of red blood corpuscles. The wall was greatly thickened and distorted by the invasion of the tumour cells, but in spite of the breaks in continuity, the wall could be followed by the remains of the elastic laminæ (Fig. 4).

The inguinal nodule was similar in appearance to the retro-peritoneal growth. No microscopic metastases were present in the sections of the lungs examined.

The interest of this tumour lies mainly in the degree of portal obstruction without the development of ascites or any obvious sign of collateral circulation. Markowitz (1937) has emphasised that, although the ascites of portal cirrhosis has long been explained on the basis of back pressure in the portal vein, ligation of the vein does not cause ascites; the latter is but a stage in the failure of liver function. This case is, therefore, of importance as an observation on a spontaneous condition which supports experimental findings. Apart from the metastases there was no liver damage.

(*b*) *Tumour of the right upper eyelid.*—The nodule was composed of solid masses of epithelial cells which invaded the dermis (Figs. 5 and 6). In places the cells were typically squamous in character while in others they were highly vacuolated and identical with sebaceous gland epithelium. The epithelium above the tumour was ulcerated. The proximity of the tumour to the position of Meibomian glands, the undoubted sebaceous character of many of the tumour cell masses and the absence of typical keratinised cell nests indicated that the neoplasm was an "adenoid carcinoma" arising from the Meibomian glands. *Adenoma sebaceum* or steatadenoma are well recognised growths in man (see Ewing, 1928,

Sutton, 1935), and several examples have been seen also in dogs, but malignant forms have not been clearly identified in either man or animals. The squamoid transformation seen in this case is not unusual in tumours arising from the dermal appendages.

In the affected right cranial cervical lymph nodes the architecture was largely obliterated by invading tumour cells though they were less differentiated (Fig. 7). The sebaceous character of the epithelium was recognisable in only a few areas. The appearances were, however, totally different from those of the retroperitoneal sarcoma. Tumour emboli filled the afferent lymphatics.

(*c*) *Testicular tumours.*—These were discrete nodules (up to 1 cm. in diameter) situated in the substance of both organs. They were composed of solid masses of large polyhedral foamy-like cells reminiscent of the hypernephroma. The tumours were not encapsulated but the seminiferous tubules round the periphery were atrophied. Such nodules are exceedingly common in dogs and in the past have been referred to as "interstitial cell tumours," although it is highly uncertain that they can be regarded as true neoplasms. They are more in the nature of nodular overgrowths comparable to the hyperplasias of normal cells commonly found in a number of organs of senile dogs such as the spleen (often termed lymphomata), the thyroid, suprarenal and liver where they form "adenoid" nodules.

Summary.

Two malignant tumours are described in a dog. One was a polymorphic or mixed-cell sarcoma arising from either the periosteum of the lumbo-sacral vertebræ or the retroperitoneal connective tissue with metastases to the liver and contiguity spread to the lesser omentum. Occlusion of the portal vein by the tumour was not accompanied by ascites, which is a valuable corollary to the experimental observation that back pressure from the portal vein is not solely responsible for the development of ascites. The other, a tumour of the eyelid, was a malignant form of *adenoma sebaceum* arising from the Meibomian glands with metastatic deposits in the cervical lymph nodes.

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FIG. 1. Primary retroperitoneal, mixed-cell sarcoma. H.E. × 300.

FIG. 2. Isolated secondary sarcoma nodule in liver with compressed and atrophied liver cells on the left. H.E. × 200.

FIG. 3. Portal vein within the liver filled with partly necrotic sarcoma tumour embolus. (*a*) Portal vein, (*b*) bile duct and (*c*) hapatic artery. H.E. × 80.

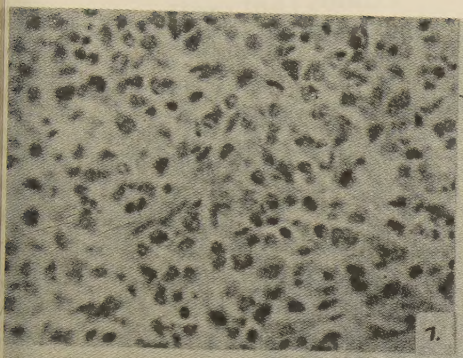
FIG. 4. Cross-section of lesser omentum showing (*a*) bile duct, (*b*) hepatic artery and (*c*) portal vein surrounded and filled by secondary sarcoma. Wilder's and van Gieson. × 2.

FIG. 5. Right eyelid with ulcerated tumour nodule close to the lid margin. To the left is the skin surface and on the right is conjunctiva. H.E. × 15.

FIG. 6. Malignant adenoma sebaceum of Meibomian glands showing squamous and sebaceous cell constituents and absence of definite cell nests. H.E. × 80.

FIG. 7. Right superior cervical lymph node containing early metastases from the eyelid carcinoma ; the epithelial cells are not so clearly differentiated as in the primary although both squamoid and sebaceous characters can be made out in places. H.E. × 80.

FIG. 8. Testes showing the presence of discrete " interstitial cell tumours." Natural size.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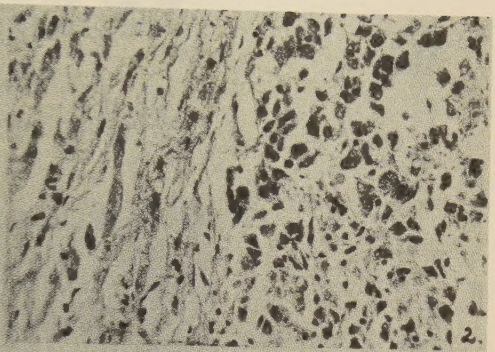
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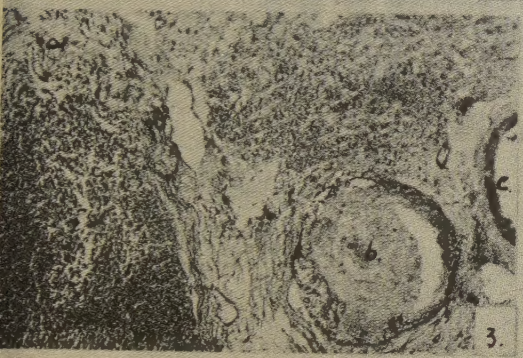
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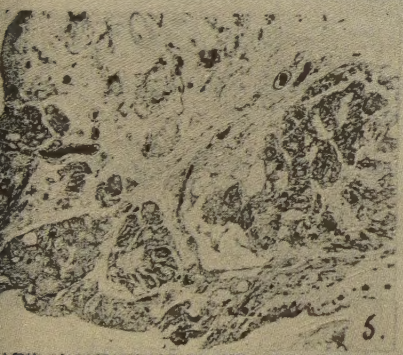
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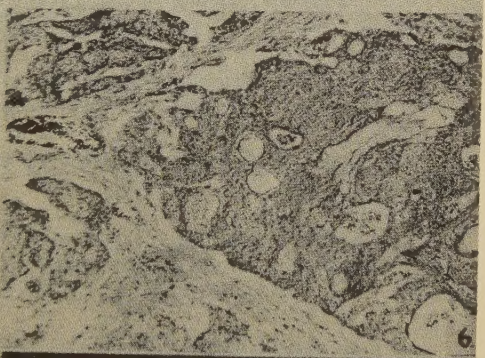
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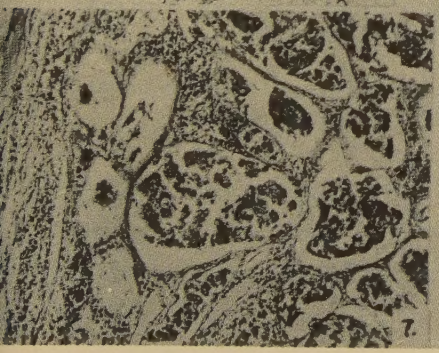
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